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EFFICACY OF ADRENAL CORTICAL EXTRACT AND OF PAREDRINE IN THE PREVENTION OF EXPERIMENTAL SHOCK FOLLOWING VENOUS OCCLUSION OF A LIMB[1]

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Recently, a method was developed in this laboratory for the experimental production of shock by occlusion of the veins in the hind leg of the dog (1). It was found that this procedure led to a state of shock followed by death in from $3\frac{1}{2}$ to 21 hours in 87 per cent of the animals. In previous reports the prophylactic and therapeutic value of two procedures were discussed: namely, the use of desoxycorticosterone acetate (DCA) (2) and the application of a cast (3). In this report we wish to present our results with adrenal cortical extract and with paredrine.

Adrenal cortical extract. Several reports have appeared suggesting that adrenal cortical extract (ACE) is beneficial in preventing shock. Perla (4) found that cortin prevented the occurrence of histamine shock in adrenalectomized rats, and Heuer and Andrus (5) found that it had a beneficial effect on blood pressure and plasma-fluid loss in dogs following the intravenous injection of extracts from obstructed bowel loops. Selye et al. (6) found cortin effective in ameliorating shock in rats following intestinal trauma. Swingle et al. (7) prevented traumatic shock in dogs by the use of adrenal cortical extracts. We therefore thought it advisable to test this extract on the type of shock which follows occlusion of the veins of the limb.

Method. As previously described, the common and internal iliac veins of one limb were ligated aseptically using a retro-peritoneal approach. This was followed by the injection of 10 to 15 cc. of a 1:20 suspension of sterile lampblack in normal saline (1). Measurements of blood pressure, heart rate, hematocrit and limb circumference were recorded at regular intervals following the operation. The dosage of adrenal cortical extract (ACE)[2] varied from 17.5 to 39.0 cc. subcutaneously—the average being 23.3 cc. (table 1). One-half of this amount was given 12 hours before the operation, and the remainder in divided doses during the first 12 post-operative hours.

Results. The results are summarized in table 1. Twelve dogs were used in this series, all but one having been given ACE preoperatively. Five animals

[1] Aided by the A. D. Nast and Emil and Fanny Wedeles Funds for Cardiovascular Research.

[2] We are grateful to Dr. D. Klein of Wilson Laboratories for generous supplies of ACE.

died within the first 24 hours after operation and necropsy indicated death due to shock. One dog died of confluent bronchopneumonia 24 hours after operation and did not appear to have developed shock. The remaining 6 animals survived.

The five animals dying of shock were found to have developed a maximum limb enlargement up to 34 per cent beyond the control size, with an increase in

TABLE 1

Effect of adrenal cortical extract (ACE) on the shock-like state following venous occlusion of the hind limb of the dog

DOG NO.	Weight	ACE, CC. SUBCUTANEOUSLY		TOTAL	SURVIVAL TIME	PER CENT INCREASE OF THIGH CIRCUMFERENCE OVER CONTROL SIZE	HEMATOCRIT	BLOOD PRESSURE	INCREASE IN WEIGHT OF LEG WITH OCCLUDED VEIN IN PER CENT OF BODY WEIGHT
		Pre-operative	Post-operative						
	kgm.				*hrs.*				
1	10.9	0	18	18	9	22	No change	Dropped	8.0
2	9.5	20	10	30	24*	9	Increased	Dropped	1.8
3	17.2	10	10	20	S†	15	Increased temporarily	Dropped temporarily	
4	8.6	10	7½	17½	7	22	Increased	Dropped	6.2
5	10.4	10	10	20	S	6	No change	Dropped temporarily	
6	11.3	10	10	20	S	14	No change	Dropped temporarily	
7	17.7	10	10	20	12–22	20	Slightly increased	Dropped	3.0
8	10.4	10	10	20	S	28	After 24 hrs. decreased	No change	
9	16.3	10	12½	22½	12	34	Increased	Dropped	5.5
10	11.3	10	10	20	S	8	Slightly increased then, after 24 hours, decreased	No change	
11	7.7	20	19	39	S	26	Slightly increased then, after 24 hours, decreased	No change	
12	10.4	20	12½	32½	6	23	Increased	Dropped	5.5

* Died of bronchopneumonia.

† Survived the operation.

weight of the edematous limb from 3.0 to 8.0 per cent of the body weight. This represents a loss of plasma fluid into the limb equivalent to from 50 to 75 per cent of the total blood volume. Survival time of these animals was no longer than that of untreated dogs. All showed a drop in blood pressure and in all but one there was a definite hemoconcentration (table 1).

Among the six dogs that survived, limb enlargement did not progress beyond 28 per cent of the control size, but this occurred after a longer interval than

in those that succumbed. The blood pressure in these animals showed a definite transitory fall in all but 3; in these exceptions no change occurred. The hematocrit showed a slight increase in 3 dogs in the first 24 hours and no change in 3. Later hemodilution occurred in three animals (table 1).

Discussion. Our results are in accord with previous work indicating that ACE exerts a beneficial effect in preventing the occurrence of the irreversible development of shock. In our ACE series of dogs, 50 per cent of the animals survived, as compared with a survival of 13 per cent of the animals in the untreated control series (1 and 2). Among the six ACE treated dogs that survived, none showed signs of developing shock.

The percentage of survivals in the ACE series was not as great as in the DCA-treated series of dogs (2). This fact may indicate that DCA is a more effective therapeutic agent than ACE, or it may be merely a question of proper dosage. The ACE contains a large number of the various steroids, in undetermined amounts, present in the adrenal cortex. Until these substances are available

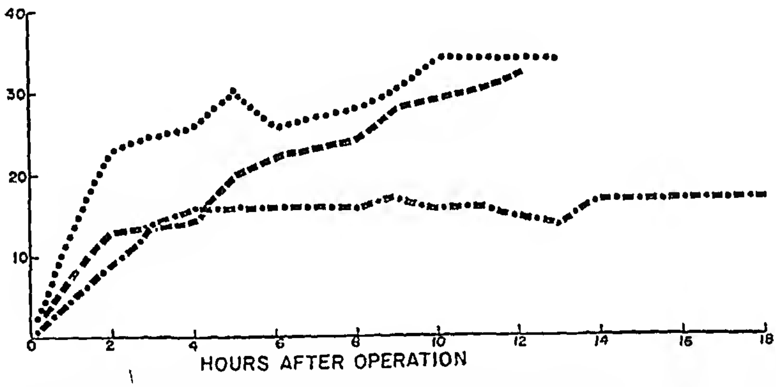

Fig. 1. This graph shows the average per cent increase in leg circumference over the control circumference following venous occlusion of the hind limb. Control series (2), dot curve. DCA series (2), dash curve. ACE series, dot-dash curve. Discussed in text.

in isolated form in sufficient amounts to permit their use in experiments such as ours, nothing can be said of their relative potency in preventing this form of shock.

The beneficial action of ACE may be either the prevention or diminution of local fluid loss into the occluded limb, or some action on the secondary mechanisms which are consequent to this decrease in circulating blood volume. In figure 1 is shown the average rate of leg enlargement of the control and primed DCA series previously reported (2), and the average rate of leg enlargement in the ACE series of the present study. This comparison shows clearly that ACE has a greater inhibiting effect on leg enlargement than DCA. There is thus no doubt that ACE diminishes local fluid loss. This action may be due in part to a decrease in capillary permeability, which has been shown to occur with ACE (8), or to some action on the osmotically active constituents of the blood, such as was indicated with DCA in our previous study (2).

Although ACE definitely inhibits local fluid loss, there is no evidence that it

may not also act on the secondary effects of decreased circulating blood volume. One possibility lies in the ability of ACE to conserve body sodium (9).

PAREDRINE. Paredrine, parahydroxyphenylisopropylamine, is a sympathomimetic drug which is more stable than epinephrine and almost entirely devoid of action on the central nervous system. Since it has a vasoconstrictor effect (10) it has been suggested as a therapeutic agent in shock to cause a sustained rise in the blood pressure (11), and thereby help maintain the flow of blood in

TABLE 2

Effect of paredrine on the shock-like state following venous occlusion of the hind limb of the dog

DOG NO.	WEIGHT	PAREDRINE ADMINISTERED			SURVIVAL TIME	PER CENT INCREASE OF THIGH CIRCUMFERENCE OVER CONTROL SIZE	HEMATOCRIT	BLOOD PRESSURE	INCREASE IN WEIGHT OF LEG WITH OCCLUDED VEIN IN PER CENT OF BODY WEIGHT
		Time after operation	Amount	Route					
	kgm.		*cc*.*		*hrs.*				*kgm.*
1	11.8	1 hr. 15 min.	1	SQ†	3	23	Markedly increased	Dropped	3.0
		2 hr. 20 min.	1	IV†					
2	10.9	35 min.	1	SQ	8¼	20	Increased	Rose and then dropped	4.1
		2 hrs.	1	SQ					
		4hrs.	1	SQ					
		7 hrs. 45min.	1	SQ					
3	8.7	3 hrs.	1	IV	6½	12	Markedly increased	Dropped	6.1
		3 hrs. 30 min.	1	SQ					
		5 hrs. 15 min.		IV					
4	11.5	1 hr. 50 min.	1	SQ	9¼	26	No change	Dropped	5.0
		4 hrs.	1	SQ					
		5 hrs. 45 min.	1	SQ					
		6 hrs. 45 min.	1	SQ					
		7 hrs. 45 min.	1	SQ					
		8 hrs. 45 min.	1	SQ					
5	11.0	1 hr. 15 min.	1	SQ	11¼	30	Markedly increased	Rose and then dropped	5.2
		3 hrs. 15 min.	½	SQ					
		7 hrs. 15 min.	1	SQ					
		9 hrs. 15 min.	1	SQ					
6	9.0	45 min.	1	SQ	5	7	Markedly increased	Rose and then dropped	3.8
		2 hrs. 45 min.	½	SQ					

* Each cubic centimeter is equal to 20 mgm. paredrine.
† SQ = subcutaneously; IV = intravenously.

the coronary vessels and other vital organs. Consequently, we tested paredrine on dogs in which shock was produced as previously described (1).

Method. The operative procedure was the same as that described above. Paredrine hydrobromide[3] was administered both subcutaneously and intravenously in doses varying from 30 to 120 mgm. per animal (table 2). The first dose was given immediately after the blood pressure began to fall, and was

[3] Generously supplied by Smith, Kline and French Laboratories.

repeated as soon as a further blood pressure drop was noted. Similar observations on heart rate, hematocrit and limb measurements were taken as in the group with ACE.

Results. In these series of six animals, all died within eleven hours after the operation; the survival time was on the average definitely shorter than that found in the control series (1 and 2). The data are summarized in table 2.

The blood pressure showed a steady decrease despite repeated rises immediately following each administration of paredrine. The limb with the occluded vein showed a rapidly increasing edema with enlargement as high as 30 per cent above the control, amounting to 6.1 per cent of the body weight. The hematocrit showed a sharp increase in all instances. The rate of fluid accumulation and the amount lost into the limb were of the same order as seen in our previous untreated series (1 and 2).

Discussion. In our experiments there was no evidence of any benefit derived from this drug although its pressor effect was apparent. This negative effect may be peculiar to the form of shock studied and need not necessarily apply to other forms. In this type of shock, elevation of blood pressure transmitted to the capillaries by raising hydrostatic pressure would tend to increase the loss of plasma fluid, and so accelerate the development of the syndrome. Such an action would be expected with paredrine in all forms of shock in which escape of plasma fluid is a prominent feature. Only when the cause of shock is extensive vasodilatation with little loss of capillary permeability could paredrine or other pressor drugs be useful, since here in addition to raising the blood pressure and increasing the driving force to feed the heart, brain, etc., it would also shrink the vascular bed, and so restore venous return to the heart (12). Since plasma fluid loss due to increased capillary permeability seems to be an important feature of most, if not all forms of shock, the aggravation of this circumstance by increasing capillary hydrostatic pressure limits the value of such pressor drugs as paredrine to emergency measures while the more important procedure of replacing the lost plasma fluids is carried out. This latter therapy and the use of cortical substances which tend to check the progress of the mechanisms which make shock irreversible seem more suitable than mere blood pressure elevation.

SUMMARY

1. Adrenal cortical extract (ACE) is a beneficial therapeutic agent in the treatment of shock following venous occlusion of a limb.
2. ACE shows a definite tendency to reduce the amount of fluid lost into the edematous limb, an action more striking than that of desoxycorticosterone acetate (DCA) alone. However, it has a less marked effect on survival than DCA.
3. Paredrine, a vasopressor substance, is of no benefit in shock in which the initiating factor is an escape of plasma fluid, since it appears to augment the escape of fluid through capillaries with impaired permeability.

We wish to express our indebtedness to Dr. L. N. Katz, at whose suggestion these studies were undertaken, for his guidance, and to Dr. K. Jochim for his suggestions in preparing this report.

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THE DISTRIBUTION OF LACTIC ACID IN HUMAN BLOOD

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Almost all of the reported observations concerning the distribution of lactic acid between blood corpuscles and plasma indicate that lactate is present at higher concentrations in plasma than in blood cells (1, 2, 3, 4). However, Devadetta (5) found that this unequal distribution obtained only when total lactate concentration was above normal, whereas at low concentrations the distribution was similar to that of the other anions of blood. In previous investigations lactic acid concentration was varied by exercise (1, 5), anoxia (2, 3), and by the addition of lactate to whole blood *in vitro* (1, 5). The present study deals with the influence of glycolysis in human blood upon the concentrations of lactic acid in water of cells and serum.

PROCEDURE. All blood samples were obtained from three normal adult subjects under basal conditions. Venipuncture was accomplished without stasis after the forearm had been immersed in hot water for about 10 minutes in order to render the blood approximately arterial in composition (6). Aerobic defibrination was carried out promptly by stirring with a glass rod. Samples of the defibrinated whole blood were taken for determination of lactic acid, glucose, cell volume and water content. The remainder was either centrifuged at once to obtain serum for determination of lactic acid and water content, or incubated at 37° for one to six and a half hours, after which whole blood samples were taken and serum obtained by centrifugation for the determinations mentioned above.

Analytical methods. Lactic acid was determined by the method of Miller and Muntz (7) as modified by Barker and Summerson (8). Analysis of desaccharinized Somogyi filtrates of whole blood and serum were carried out in triplicate. No antiglycolytic agents were utilized in the procedures. It was found that no significant rise in blood lactate occurred during the period required for defibrination. Furthermore, lactic acid concentrations in defibrinated blood allowed to stand at room temperature for 15 to 20 minutes before precipitation of proteins never exceeded 14 mgm. per cent and often fell within the range of values for blood precipitated immediately.

Blood glucose was determined on Somogyi filtrates (9) by the method of Benedict (10).

Cell volume was measured with Daland hematocrit tubes as recommended by Eisenman, Mackenzie and Peters (11).

[1] This article represents work done in fulfilment of the thesis requirement for the degree of Doctor of Medicine at Yale University School of Medicine.

Water content was taken as the loss of weight of an aliquot of whole blood or serum dried to constant weight at 97.5°.

Calculations. Concentration of lactate in cells was calculated from concentrations in whole blood and serum by the formula:

$$L_c = \frac{L_b - L_s(1 - V_c)}{V_c}$$

TABLE 1

The distribution of lactic acid between cells and serum during glycolysis

EXPERIMENT NUMBER	SUBJECT	DURATION OF INCUBATION	CELL VOLUME		LACTIC ACID IN WHOLE BLOOD		FINAL LACTIC ACID IN WATER OF		DISTRIBUTION RATIO (1):(2)
			Initial	Final	Initial	Final	Cells (1)	Serum (2)	
		hours	*per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	
1	D. D.	1	51.9	47.0	9.6	18.6	8.0	31.9	0.3
2	D. D.	1	49.4	47.5	7.0	21.0	14.5	32.9	0.4
3	D. D.	1	49.6	46.7	7.5	26.0	24.2	35.9	0.7
4	E. B. D.	1	43.1	38.7	7.8	20.4	20.3	25.7	0.8
5	J. D. R.	1	45.0	42.3	6.8	25.0	27.2	31.8	0.8
6	J. D. R.	1	41.0	42.1	9.7	28.0	31.6	34.6	0.9
7	E. B. D.	1	41.9	38.6	5.2	19.0	22.0	22.6	1.0
8	D. D.	1	51.1	47.9	6.5	23.2	27.9	28.4	1.0
9	J. D. R.	1	45.9	44.0	7.5	21.0	23.8	26.4	0.9
10	D. D.	1	49.9	48.0	7.6	25.0	36.8	25.5	1.4
11 A	J. D. R.	5		50.7		70.6	60.4	104.5	0.6
11 B	J. D. R.	6½		50.1		62.8	22.1	119.5	0.2
12 A	J. D. R.	5		47.0		70.0	69.9	93.1	0.7
12 B	J. D. R.	6½		50.9		80.4	64.2	122.0	0.5
13 A	D. D.	5		57.3		62.0	39.6	112.4	0.4
13 B	D. D.	6½		58.6		65.6	43.9	119.4	0.4
14*	D. D.	5	47.7	50.2	6.3	74.0	71.6	101.9	0.7
15*	D. D.	5	45.7	50.2	10.3	82.8	90.0	104.7	0.9
16*	J. D. R.	5	51.0	57.4	8.0	84.4	74.3	129.9	0.6
17*	J. D. R.	5	51.7	53.3	12.0	80.4	59.0	129.5	0.5

* Glucose added before incubation. Initial glucose concentrations: experiment 14, 172; experiment 15, 163; experiment 16, 199; experiment 17, 207 mgm. per cent.

in which L_c, L_b, and L_s are lactate concentrations in cells, whole blood and serum, respectively, and V_c is the cell volume.

The concentration of water in cells was calculated from the water of whole blood and of serum by an analogous formula.

RESULTS. Ten measurements of the lactic acid concentration in blood drawn under basal conditions and deproteinized immediately after defibrination, gave values varying from 5.2 to 9.7 mgm. per cent. These values are in good agreement with basal values reported in the more recent literature. In 5 of 6 instances in which lactic acid concentrations in water of cells and serum were compared under basal conditions, the two concentrations were identical within the limits of error of the analytical procedures. In a single experiment the concen-

tration in cell water was 16.5 mgm. per cent, while that in serum water was only 11.6 mgm. per cent. With this single exception, lactic acid was found to be evenly distributed through the water of cells and serum under basal conditions. These observations confirm those of Devadetta (5).

Concentrations of lactic acid in cell water and serum water after glycolysis has been allowed to proceed at 37° for 1, 5 and 6½ hour periods are given in table 1. After glycolysis of one hour's duration a tendency for serum lactate to exceed the concentration in cells is already apparent and this inequality of distribution is more marked and more consistent at the end of the longer periods.

Increments in whole blood lactate concentration and decrements in blood glucose concentration during incubation periods of 1 and 5 hours are compared in table 2. Except in experiment 7, in which an unusually small decrease in

TABLE 2

The relation of lactic acid production to glucose destruction in blood during glycolysis

EXPERIMENT NUMBER	SUBJECT	DURATION OF INCUBATION	DECREASE IN BLOOD SUGAR	INCREASE IN BLOOD LACTATE	GLUCOSE CONVERTED TO LACTIC ACID
		hours	*mgm. per cent*	*mgm. per cent*	*per cent*
1	D.D.	1	15	9.0	60
2	D. D.	1	24	14.0	58
3	D. D.	1	24	18.5	75
8	D. D.	1	24	16.7	70
10	D. D.	1	19	17.4	94
5	J. D. R.	1	30	18.2	61
9	J. D. R.	1	23	13.8	60
6	J. D. R.	1	24	18.3	76
4	E. B. D.	1	14	12.6	90
7	E. B. D.	1	8	13.8	174
14*	D. D.	5	81	67.7	83
15*	D. D.	5	85	72.8	86
16*	J. D. R.	5	107	76.4	71
17*	J. D. R.	5	95	68.4	72

* Glucose added before incubation.

blood glucose was observed, only 58 to 94 per cent of the glucose which disappeared could be accounted for as increased lactate.

A decrease in cell volume was frequently observed at the end of 1 hour's incubation (table 1). In experiment 6 an insignificant rise was observed (1.1 vols. per cent). In experiments 2, 9 and 10 a probably significant fall occurred (2.7 to 4.9 vols. per cent). After 5 hours' incubation no significant net change in cell volume occurred in two experiments whereas in two others cell volume increased by 4.5 and 6.4 vols. per cent.

DISCUSSION. Blood lactic acid at the normal basal value is equally distributed through the water of cells and serum. When, however, the lactic acid concentration is raised, whether by addition of lactate (1, 5), exercise (1, 5), anoxia (2, 3), or by glycolysis, the concentration in serum water almost always exceeds

that in cell water. This unequal distribution cannot be attributed to restraint of diffusion exerted by the serum colloids since lactate added to suspensions of blood cells in saline is distributed just as it is in whole blood (5). Devadetta (5) has also shown that lactate ion can cross the corpuscular membrane in either direction. The validity of these observations is supported by the ability of lactate to leave the cells and to enter the serum during glycolysis. The partition of lactate cannot be dependent upon the glycolytic process since procedures which prevent glycolysis, such as addition of fluoride or washing the blood corpuscles, do not alter the distribution of lactic acid (5). It is not improbable, however, that the glycolytic process does modify lactate partition to some extent. Whereas in Devadetta's experiments, in which glycolysis played no role, a good correlation existed between the distribution ratio of lactate and the total lactate concentration, in the present study there is only a fair correlation between these variables. Furthermore, in one experiment (no. 11) a large amount of lactate entered the serum from the cells in the period just after glycolysis was completed and without any further rise in total lactate. Since it is known that other ions (12) are transferred during glycolysis, it seems not improbable that the lactate partition expected on the basis of concentration alone might be altered in response to the metabolic requirements of the cells.

Failure to recover lactic acid in sufficient quantity to account for the decrease in blood sugar during glycolysis may be in part due to oxidative metabolism of glucose by the white blood cells. However, the amount of glucose which could disappear by this route is probably too small to account for the entire deficit observed. Furthermore, quantitative conversion of glucose to lactic acid may not occur even when oxidative processes are inhibited (13).

This disparate distribution must have some bearing on data dealing with lactic acid equilibria in the living organism. Obviously, if there is such an equilibrium it must be between the concentrations in the tissues and the plasma, not whole blood. After exercise or in other conditions in which lactic acid is elevated, its concentration may be far lower in whole blood than in plasma.

Whether other cells behave like blood cells is not known. Ghaffar (14) found that lactate added to frog muscle tissue distributed itself through a volume of fluid approximating that of the extracellular space only. Lactic acid produced in the muscle appeared to diffuse out freely; but even when none was added, the concentration was greater in extracellular fluid than in the muscle cells. Devadetta (15), in similar experiments, estimated that lactic acid diffused into all the water of muscle. Newman (16) found that the concentrations of lactic acid in blood and muscle taken from rats both before and after exercise differed by no more than the experimental error. Gesell et al. (2) and Eggleton and Evans (4) have analyzed plasma and muscles under various conditions. Although on the average concentrations in the two media agreed, in individual observations there were divergences in both directions. There was some tendency for the concentrations to be greater in the plasma when lactic acid was elevated, the disparities in some instances being too great to be attributed to differences in the amounts of water in the two media. Determination of the distribution of lactate in the

tissues is an extremely difficult matter, since the acid is so ubiquitously produced and so continually removed, and since production does not cease when the tissue is abstracted for analysis. Since, in the animal lactic acid originates in the muscle, if it enters the blood by a simple process of diffusion, it should, as it rises, be more concentrated in muscle than in plasma. Those experiments in which the concentration gradient is in the opposite direction, from plasma to muscle, must, therefore, be given extra weight.

Danowski (12) in experiments on the transfer of potassium between blood cells and plasma found that at the end of 5 hours' incubation the volume of the cells had not changed. In a single instance he noted some contraction of the cells after 2.5 hours. In the present experiments the volume of the cells decreases distinctly in the course of the first hour, but has been again restored at the end of 5 hours. The reactions associated with this transitory transfer of water remain to be discovered.

SUMMARY

The distribution of lactic acid between blood cells and plasma during spontaneous glycolysis has been investigated. In the blood of humans at rest in the post-absorptive state, lactate is evenly distributed through the water of cells and serum. As the concentration of lactic acid rises with glycolysis, the ratio, lactic acid in water of serum: lactic acid in water of cells, steadily increases. This is in agreement with Devadetta's observations of the distribution of lactic acid at rest, during exercise and after the addition of lactate.

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INFLUENCE OF PROPRIOCEPTIVE VAGAL AFFERENTS ON PANTING AND ACCESSORY PANTING MOVEMENTS IN MAMMALS AND BIRDS

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Since the important original investigations of Hering and Breuer in 1868 on the influence of the vagi on respiration, subsequent evidence has increased our knowledge of their significance. The modern concept is essentially that of a drive mechanism which together with the drive of higher centers (e.g., the "pneumotaxic" center of Lumsden) maintains respiratory rhythmicity. The vagal effects have been shown to be essentially inhibito-inspiratory or excito-expiratory resulting in a more shallow and consequently faster type of breathing. The biological significance of such an arrangement must be prevention of excessively great variations in alveolar oxygen and carbon dioxide pressures thus approaching more nearly a condition of homeostasis.

Among mammals and especially in birds vagotomy results in decreased breathing rates. Vagotomy in the fowl results in an alarming decrease in rate of pulmonary ventilation such that the bird becomes cyanotic. Inspirations occur as infrequently as four per minute. During successive days the rate increases somewhat. Cocainization of the vagi produces effects identical with vagotomy.

It has been stated (Pitts, Magoun and Ranson, 1939) that rhythmicity (in the cat) is imposed upon the medullary centers by two bilateral mechanisms: one a vagal mechanism, the other a pneumotaxic mechanism, the center for which is located in the upper few millimeters of the pons. If the connections of both these mechanisms with the respiratory centers are lost, respiratory rhythmicity is interrupted and in its place a tonic, maintained inspiration ensues. If, however, there remains one vagus intact, or a part of one descending pneumotaxic connection with the respiratory center, its action remains rhythmical. Hong and Nicholson (1942) report that in decerebrate dogs with mid-pontine transection intermittent central vagal stimulation may cause normal rhythmic breathing at a frequency corresponding to that of the stimulation. Nicholson and Hong (1942) showed that section through the middle or upper pons in the anesthetized (morphine-urethane, pentobarbital or ether) dog commonly resulted in respiratory arrest and death. In the non-anesthetized dog, however, similar section produced only deep held inspirations with superimposed rhythmical respiration at the normal rate. Pitts (1942) also shows that transection of the brain through the middle or lower pons plus bilateral vagal section is followed by apneusis. Stella (1939) has shown that the pneumotaxic connections with the apneustic center are bilateral and are mainly homolateral.

We have found that panting can occur in mammals after bilateral vagotomy and in fact appears entirely normal (fig. 1, A). If one vagus is cut (or cocainized)

in the panting bird no visible change occurs, but if the other vagus is then sectioned (or cocainized) panting stops at once (fig. 1, B, C). Even though the body temperature is well above the panting threshold, panting immediately

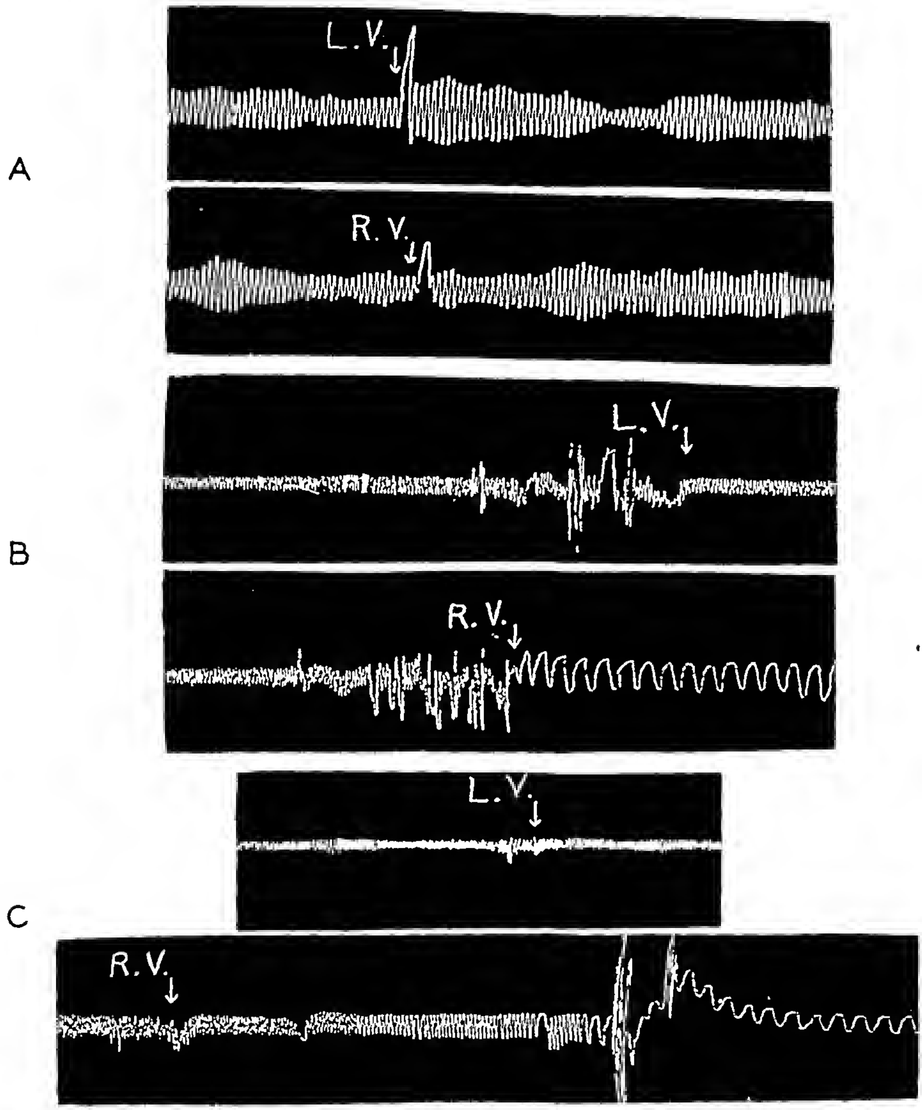

Fig. 1. A. Record of panting in the rabbit. Section of left and right vagus indicated by arrows. B. Panting in the chicken. Section of left and right vagus indicated by arrows. C. Panting in the chicken. Application of cocaine hydrochloride (1.0 per cent solution) to left and right vagus shown by arrows. Note no change in rate after cocainization of left vagus but an effect taking place quantitatively as separate fibers of the right vagus become blocked.

ceases when the second vagus is sectioned. Nor does it matter in which order section of the two vagi occurs, either left or right may be sectioned first with no apparent effect on the panting rhythm. This raises an interesting speculation. Why should no change in panting rate result from unilateral vagotomy while

abrupt cessation results from double vagotomy? It appears that either vagus is more than an ample pathway for the Hering-Breuer fibers and likewise that both are functioning submaximally as regards the number of fibers at all times. One might imagine an analogue to a group of wires in a cable supplying an electric lamp. If half of the wires are cut the lamp will continue to glow without reduced intensity if the remaining wires are still ample to conduct the necessary current.

Examination of figure 1, B will show the continuation of panting with no reduced rate after section of the left vagus but a prompt abolition after the remaining vagus has been cut. No such effect can be found in the panting rabbit either with cocainization or surgical severance of the vagi.

During panting in birds and mammals there occur certain accessory movements together with salivation. In the rabbit the nose-flap can be seen to move with each breath. Tracheotomy has no effect on this motor response. In the bird accessory panting movements consist of opening and closing the beak and raising and lowering the tongue. Tracheotomy and the resulting "sham panting" do not influence those motor responses. Salivation is more apparent in the tracheotomized panting mammal or bird due to the absence of the air-stream in the naso-pharynx.

At attempt to study the effect of the stretch receptors of the lungs on the panting rhythm in birds and mammals was carried out by artificial distention of the lungs via a tracheal cannula and by artificial respiration induced by bellows action at a rate slower than that of panting. Emphysema was produced by attaching a rubber hose to the tracheal cannula and forcing air into it during panting thus stimulating the stretch receptors during polypneic breathing. With the rabbit distension of the lungs caused temporary cessation of panting. As soon as the pressure was relieved the polypneic rhythm returned. It should be mentioned that if the distention of the lungs was maintained for a length of time the panting movements returned probably due to an accumulation of carbon dioxide or to an increase in sensitivity of the centers or to both.

With the non-anesthetized duck distention of the lungs either by a steady positive pressure or intermittently by bellows action caused no change in the panting rhythm. However, in the duck anesthetized with a barbiturate (sodium amytal) distention of the lungs during panting caused slowing of the polypneic rhythm which became progressively slower with maintained distention until complete apnea occurred. This points to the fact that barbiturate narcosis can modify the normal respiratory regulation which has been reported by others (Beecher and Moyer, 1942; Nicholson and Hong, 1942) and was shown by us in previous work (Randall and Hiestand, 1939). It shows that during amytal anesthesia the relationship of the stretch receptors to the respiratory centers, e.g., the panting center, is interfered with.

By comparison of the bird and mammal we thus see that distention of the lungs of the rabbit is more effective in reducing the rate of polypneic breathing than in the bird but at the same time we must acknowledge the greater significance of the proprioceptive vagal drive of the bird because panting is impossible

without it. It seems paradoxical that emphysema should not slow or abolish breathing in the bird if the vagal drive here is more powerful. One imagines the "vagal drive" as an inhibito-inspiratory mechanism (and possibly inhibito-expiratory) that depends upon stretch (or collapse) of the lungs for its stimulus. Why then should panting continue unabated in the rabbit and cease altogether in the bird after vagotomy? It must be that the rabbit depends upon a panting drive from a "higher" brain center entirely whereas the bird depends mainly upon the vago-proprioceptive drive or vago and pneumotaxic drives.

In the mammal section of one vagus reduces the breathing rate; section of the remaining vagus reduces it still further, and yet the vagotomized rabbit can pant normally. In the bird section of one vagus reduces the breathing rate considerably more than in the mammal; section of the other vagus almost produces respiratory arrest. The vagotomized bird can not pant. If the vagal drive in birds is so much greater than in mammals why does emphysema reduce breathing in the mammal but not in the bird? Perhaps the stimulus of emphysema is entirely different from the normal lung-stretch breathing stimulus, or possibly different types of receptors exist in the lungs.

CONCLUSIONS

1. Stimulation of the pulmonary stretch receptors by emphysema in the rabbit during panting progressively reduces the panting rate and results in apnea if prolonged.
2. No such effect occurs in the non-anesthetized bird during panting.
3. Under barbiturate anesthesia stimulation of the stretch receptors results in slowing of the panting rate in birds similar to that of the non-anesthetized mammal.
4. Section (or narcotization) of either or both of the vagi has no effect on panting in mammals.
5. Section (or narcotization) of either of the vagi of birds has no effect on panting.
6. Section (or narcotization) of both vagi of birds immediately abolishes panting regardless of the order of sectioning.
7. The vagal proprioceptive drive is relatively more significant in respiratory regulation in birds than in mammals.

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RELATION BETWEEN PULMONARY VENTILATION AND OXYGEN CONSUMPTION AFTER EXERCISE

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The cardio-respiratory system is the most important limiting factor in the performance of strenuous muscular exertion by normal men. Measurements of its functions have given a reasonably complete analysis of the mutual relationships of its component parts during exercise (see, for example, the work of Boothby (3) and of Bock, Dill and their colleagues (2)). Somewhat less attention has been paid by physiologists to the cardio-respiratory functions in recovery after exercise than during exercise. For this reason, we decided to study in recovery after exercise the cardiac output, the blood lactate, oxygen consumption, pulmonary ventilation and pulse rate in order to find out if it is possible to relate them one with another and at the same time to see whether the individual variations from subject to subject are indicative of variations in general physical fitness. The few reports in the literature pertinent to this particular study will be mentioned in the discussion of our results.

Experimental procedure. Two types of experiments were carried out, differing in the grade of exercise chosen and the measurements made during recovery.

1. The subject either ran on a motor-driven treadmill at 7 miles per hour and 8.6 per cent grade until exhausted or else walked at 3.5 miles per hour on the same grade until he reached a steady state. He then sat quietly during recovery. During the entire experiment the ventilation was measured by collecting the expired air in a large Tissot gasometer and the oxygen consumption was measured from samples drawn at frequent intervals from a mixing chamber inserted between the subject and the gasometer.

2. In the second type of experiment the exercise was always a walk at 3.5 miles per hour with the grade at 7.5, 8.6 or 9.5 per cent. Ventilation and oxygen consumption were determined by collection and analysis of expired air at only three points: *a*, during the steady state; *b*, between three and five minutes of recovery; *c*, at 20 minutes in recovery. At the same time the cardiac output was measured by Grollman's (5) acetylene rebreathing method, and the lactate in the blood from a finger prick was determined by Edwards' (3) modification of the method of Friedemann, Cotonio and Shaffer (4).

Results and discussion. Figures 1, 2 and 3 depict the blood lactate, the pulmonary ventilation, the pulse rate, the cardiac output, and the oxygen consumption following two grades of exercise. In each case the scales are

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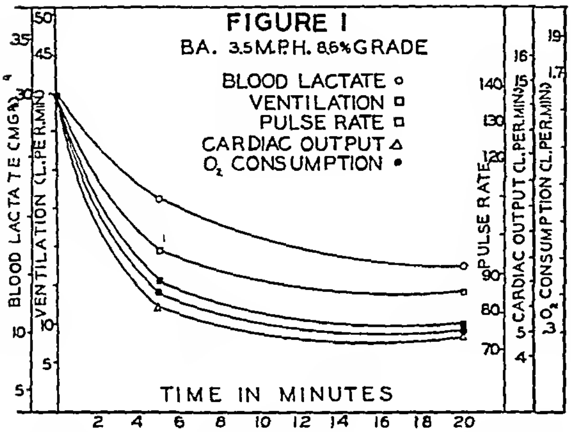
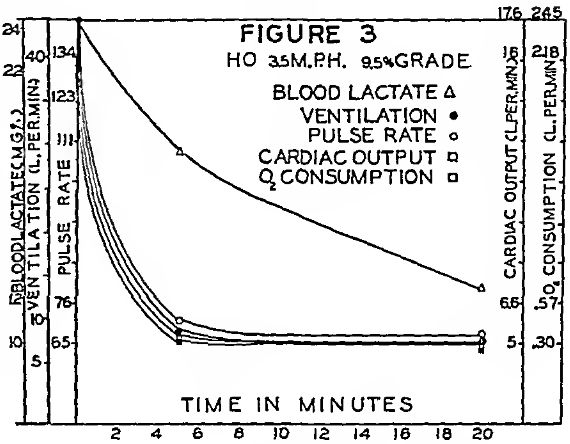
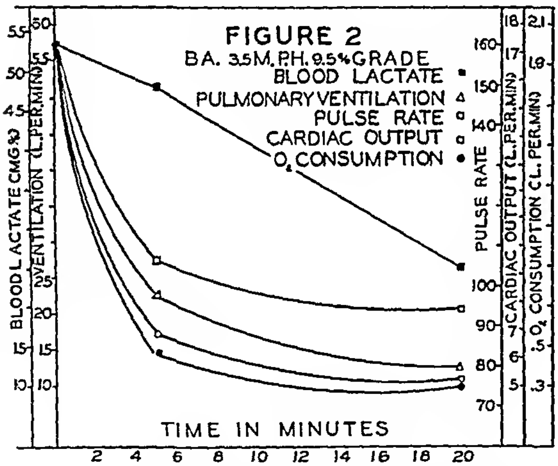
chosen and placed so that all the curves start at a common point which represents the value of that variable in the steady state of the exercise. In this way, although actual values are plotted, the same type of curve is obtained as if the measurements were calculated as percentages of the values at steady state. It will be seen that all of the variables decrease, and in order of slowness fall in







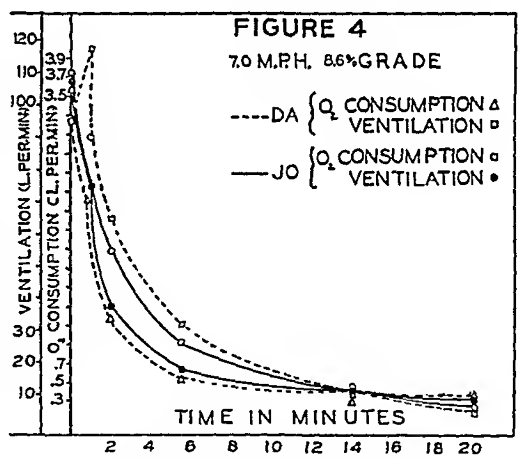


Fig. 1. Oxygen consumption, pulmonary ventilation, blood lactate, and cardiac output in subject *Ba* after walking 12 minutes at 3.5 miles per hour and 8.6 per cent grade.

Fig. 2. The same measurements on the same subject walking at 3.5 miles per hour and 9.5 per cent grade.

Fig. 3. The same measurements on the subject *Ho* after walking 12 minutes at 3.5 miles per hour and 9.5 per cent grade.

Fig. 4. Oxygen consumption and pulmonary ventilation of subjects *Da* and *Jo* after running to exhaustion at 7.0 miles per hour and 8.6 per cent grade.

the following order: lactate, ventilation, pulse rate, oxygen consumption and cardiac output. The last two follow one another very closely, which finding agrees with that of Bansi and Groscurth (1) on normal men as contrasted with cardiac patients. Likewise the relations between pulse rate and oxygen consumption are in confirmation to the work of Lythgoe and Pereira (7).

The special relation between ventilation and oxygen consumption can be

shown best by examining the ratio oxygen consumption/pulmonary ventilation, the ventilatory efficiency. Table 1 shows repeated measurements of this value

TABLE 1

Ventilation and oxygen consumption during recovery after 15 minutes' walking uphill at 3.5 m.p.h. and 8.6 per cent grade

SUBJECT	TIME IN RECOVERY	PULMONARY VENTILATION	OXYGEN CONSUMPTION	VENTILATORY EFFICIENCY	BLOOD LACTATE
	minutes	*l. per min. S.T.P.*	*l. per min. S.T.P.*	OXYGEN CONSUMPTION / PULMONARY VENTILATION	*mgm. in 100 ml. of blood*
De	0– 1	34.92	0.701	20.1	40
	1– 3	20.02	0.404	20.0	
	3– 5	18.62	0.402	21.6	32
	5– 8	16.60	0.391	24.0	
	8–10	15.36	0.350	22.8	
	10–12	13.00	0.341	26.2	
	12–15	13.65	0.340	24.9	22
Ho	0– 1	14.88	0.495	33.2	18
	1– 3	13.48	0.430	31.9	
	3– 5	12.00	0.405	33.7	16
	5– 8	10.40	0.313	30.0	
	8–10	9.50	0.280	29.6	
	10–12	8.52	0.260	30.6	
	12–15	7.00	0.260	37.2	12

TABLE 2

Individual variations in ventilatory efficiency compared with physical fitness for hard work

SUBJECT	INDEX OF PHYSICAL FITNESS*	VENTILATORY EFFICIENCY OXYGEN CONSUMPTION/PULMONARY VENTILATION	
		Ten minutes after moderate exercise	Ten minutes after exhausting exercise
Ho	95	41.5	28
Sw	88	41.3	
Do	78	39.8	
Jo	73		25.6
Wit	63		24.6
Mo	55	35.7	24.3
Fo	52	35.7	24.1
Fa	47	33.3	24.1
He	41		24.3
Ba	22	28.6	21.7
Da	22		20.9
De	16†	21.6	

* According to the test of Johnson, Brouha and Darling.
† Measured by other criteria.

in recovery after moderate exercise, and figure 4 the pulmonary ventilation and oxygen consumption of two subjects after exhausting exercise. The dissociation between the curves for ventilation and oxygen consumption is more marked

after severe than after moderate exercise, and as shown in table 1, is associated with differences in blood lactate. The results lend themselves to the interpretation that ventilation is maintained relatively higher than oxygen consumption by increased acidity of the blood.

On examining the data for different subjects, considerable variation is found in the ventilatory efficiency after work is over which might logically be related to the physical fitness of the individuals. Table 1 shows that for a subject (De) known to be in poor physical condition, the ventilatory efficiency is poor at all times after moderate exercise, and that for a subject known to be in good physical condition (Ho) it is good throughout. Table 2 lists twelve subjects in order of their physical fitness for hard work as measured by the test of Johnson, Brouha and Darling (6). It will be seen that in this small number of subjects there is a good correlation between the subject's general physical condition for hard work and his ventilatory efficiency in recovery after exercise. Estimation of ventilatory efficiency is hardly practicable for routine testing of physical fitness, but might be useful clinically in following the progress of patients with pulmonary and cardiac disease.

SUMMARY

1. The relations among pulmonary ventilation, oxygen consumption, cardiac output, pulse rate, and blood lactate have been studied in the recovery period after exercise.

2. The rates of decrease of oxygen consumption and cardiac output are almost the same; the rate of decrease of the pulse rate is slightly slower; that of pulmonary ventilation is even slower; that of the blood lactate is the slowest of all.

3. It is suggested that the deviation of the curve for ventilation is caused by a continued stimulus to ventilation in the blood stream, as shown by the blood lactate.

4. The ventilatory efficiency as expressed by the ratio oxygen consumption/pulmonary ventilation is less after exhausting than after moderate exercise, and shows considerable variation from subject to subject after the same amount of work.

5. In twelve subjects, the ventilatory efficiency correlates well with their established general physical fitness for hard muscular work.

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METABOLIC EFFECTS OF LOCAL ISCHEMIA DURING MUSCULAR EXERCISE

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The method of Jarisch and Gaisböck (11) of studying the effect of ischemia of the legs and arms furnishes a useful technique for measuring various aspects of metabolism. These authors as well as Asmussen, Nielsen and Christensen (1) studied its effect upon oxygen consumption.

The same technique is utilized in this paper to measure at the same time the changes in lactic acid. On the general subject of lactate metabolism, Hill (9) and associates first studied intensively the lactate concentration in blood following muscular exercise, the rate of its removal from the blood stream and its relation with oxygen consumption and oxygen debt. Margaria, Edwards and Dill (14) established that the removal of lactate from the body of a man resting after exercise is proportional to the amount that is present at any given time. Newman and his associates (17) observed that the rate of removal of lactate in exercise increases in almost direct proportion to the metabolic rate up to some critical level, different for each subject. Recently, Robinson (18) has studied the rate of removal of lactate in exercise and rest and its relation to the oxygen debt, with subjects performing successively decreasing amounts of work and has found that the rate of removal of lactate is higher during work and that the extra oxygen consumption for lactate removal is lower than at rest.

METHODS. A. *Analytical methods.* Analysis of the samples of expired air was made with a Haldane gas analysis apparatus. Blood lactates were measured by Edwards' (3) modification of the method of Friedemann, Cotonio and Shaffer (7).

B. *Experimental procedure.* The experiments consisted in cutting off the limb's circulation while muscular work was being carried out, and in relieving the pressure after a period of work in order to liberate into the body the metabolites produced locally by the limb's activity. In effect, therefore, the conditions were analogous to making a sudden injection into the general circulation of these accumulated metabolites, making possible a study of lactate diffusion and removal, and its relation with oxygen debt.

Two fundamental types of experiments were performed:

1. *Experiment allowing recovery at rest.* The subject, well trained for the procedure, walked on a motor-driven treadmill at 3.5 m.p.h., 8.6 per cent grade, until a steady state was reached (usually within 12 min.). Then, by means of sphygmomanometer cuffs, the circulation of both legs was rapidly cut off just at the distal insertion of the gluteal muscles.

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After an appropriate interval, usually about one and one-half minutes, the pressure was instantaneously relieved and the subject rested for 20 minutes or longer. Samples of capillary blood and of expired air were taken before, during and after the ischemia.

2. *Experiment allowing recovery during continued walking.* The second type of experiment was carried out in the same way, the only difference being that after release of the ischemia, the subject, instead of resting, continued to walk on the treadmill at the same rate and grade as before.

C. *Determination of the muscle mass.* The muscular mass was estimated by measuring the volume of water displaced by immersion of the leg. We have assumed that 70 per cent of the mass of the legs is muscle (Froshe and Fraenkel, and Vierords Tabellen cited by Jarisch and Gaisböck (11)) and that the muscle of the legs accounts for 43 per cent of the total muscle of the body.

TABLE 1

Effects of ischemia of both legs during walking uphill at a grade of 8.6 per cent

SUBJECT	SPEED OF WALK	CONDITION OF CIRCULATION OF LEGS	PULMONARY VENTILATION	OXYGEN CONSUMPTION	R.Q.
	m.p.h.		*l. per min.*	*l. per min.*	
Bar	3.5	Normal	43.7	1.525	0.90
		Ischemic	38.2	1.146	0.92
		First min. after ischemia	55.4	1.950	1.12
Bar	2.4	Normal	36.6	1.320	0.84
		Ischemic	33.0	1.062	0.82
		First min. after ischemia	50.0	1.750	1.19
Con	3.5	Normal	48.0	2.425	0.89
		Ischemic	43.0	1.680	0.90
		First min. after ischemia	70.5	3.250	1.09
Con	2.4	Normal	38.6	1.941	0.85
		Ischemic	35.4	1.344	0.95
		First min. after ischemia	52.6	2.250	1.10

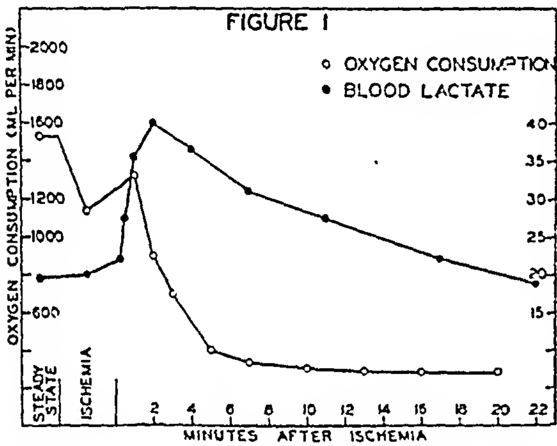
Results and discussion. Subjectively, the subjects noticed during the ischemic period, progressive difficulty in walking because of numbness, stiffness, and at the end of the period, usually cramps, inability to move and pain in almost every muscle of the leg. Upon relief of the ischemia the subject noticed within a few seconds the following symptoms in order of appearance: *a*, increased ventilation; *b*, palpitation of the heart; *c*, outbreak of profuse cold sweating. These symptoms reached their greatest intensity usually within fifteen seconds and then rapidly subsided.

I. *Oxygen consumption and ventilation.* The effects of ischemia during walking at two different rates are shown in table 1 for two subjects. In agreement with the work of Asmussen (1) there was during ischemia a diminution of the oxygen consumption. In addition we found a diminution of ventilation, and no change in the R.Q. During the first minute after ischemia there was a large increase in ventilation, oxygen consumption and R.Q.

The oxygen consumption of the leg muscles was calculated according to the method of Asmussen, Nielsen and Christensen (1), who assume that the difference between the oxygen consumption in the steady state and in the ischemic state represents the oxygen consumption of the legs. For one subject walking at 3.5 m.p.h. and 8.6 per cent grade the oxygen consumption of the leg muscles was thus calculated to be 38.7 cc. per kilogram and minute, and per kilogram-meter of work done by the body was 0.096 cc. per kilogram of leg muscle.

The cause of the sudden change in ventilation and oxygen consumption upon release of ischemia is apparently the sudden influx into the general circulation of the metabolites of the working ischemic muscles.

The relations among ventilation, oxygen consumption and blood lactate are shown in table 2 and in figures 1 and 2 for the period following release of ischemia. Typical experiments are shown in which the subject sat down im-



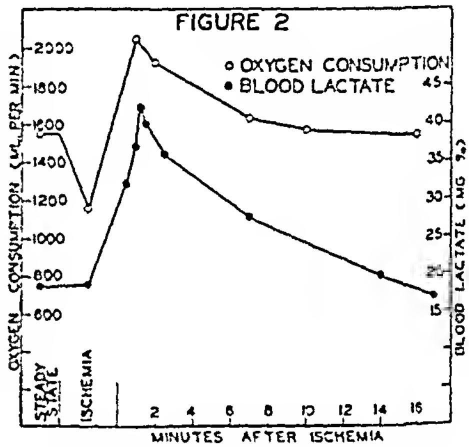


Fig. 1. Oxygen consumption and blood lactate in typical experiment on subject *Ba* during and after release of total ischemia of both legs. The subject began to rest at the same time as ischemia was relieved.

Fig. 2. The same measurements as in figure 1. The subject continued walking after release of the ischemia.

mediately after release of the ischemia and in which the subject continued to walk. The increases in ventilation and in oxygen consumption are proportional to the amount of lactate released into the general blood stream. In the walking experiments, the ventilation and oxygen return to their control levels within ten minutes after release of the ischemia; in the resting experiments, within twenty minutes.

Further discussion of the changes in ventilation in relation to the work of Harrison (8) will be reserved for another paper.

II. *Production of lactate by the muscles during activity.* After release of the ischemia, lactate rapidly diffused into the blood stream as shown in figures 1 and 2. Its maximal value after the release of ischemia was reached in about $1\frac{1}{2}$ minutes when the subject continued walking, and in about 2 minutes when he stopped walking at the time ischemia was relieved. The time spent for lactic acid to reach its equilibrium in blood can be attributed to the time neces-

sary for diffusion of the lactic acid from muscles to blood and into the idle tissues. This period ought to be shorter in exercise than in rest, because of the increased blood flow.

We have computed the quantity of lactate produced by a given muscle mass during a given amount of work making an approximation after Jarisch and Gaisböck (11) of the mass of muscle with the circulation arrested and the increase of lactic acid in the blood when the circulation was restored. For this computation we have assumed the lactate to be uniformly distributed in the fluid of the body, as shown by Margaria and Edwards (13) and by Newman (16). The lactate production under the conditions of our experiments increases with faster rate of work (fig. 4) and the muscular mass (fig. 5).

III. *Removal of lactate.* Data concerning the removal of lactate from the blood stream at rest and during work are shown in table 2 and in figures 1 and 2. In agreement with the equation of Margaria and Edwards (13) three facts

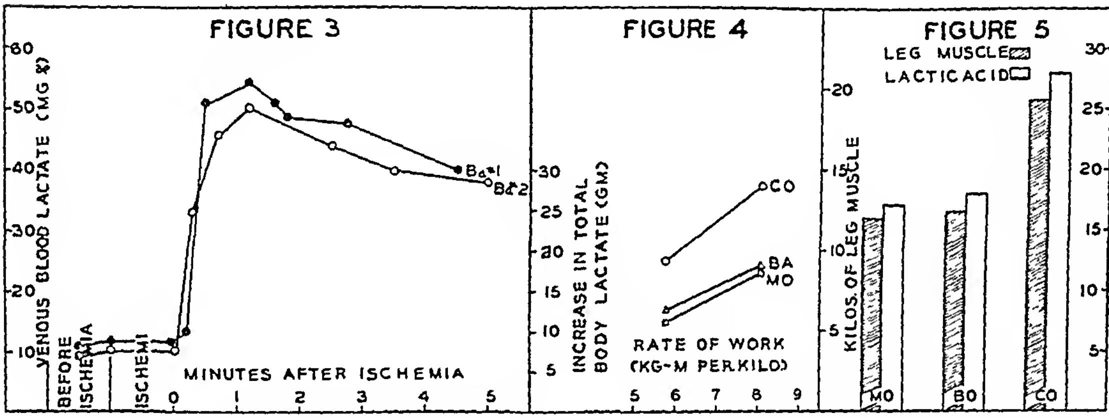


Fig. 3. Diffusion of lactate into the local venous blood before, during and after total ischemia of the exercising arm.

Fig. 4. The lactate production during ischemia of the legs in relation to rate of work.

Fig. 5. The total lactate production in relation to the bulk of the leg muscles.

worthy of consideration are: *a*. The lactate removal is an exponential function expressed approximately by the equation, $y = ae^{-kx} + b$; where a is a constant equivalent to the maximal lactate value minus the constant b; b is the lactic acid concentration at rest in the resting experiment and also represents the lactate at steady state in the walking experiment; k is the velocity constant that expresses how a approaches b through time "t". *b*. The value of k is much larger when the lactate is removed during continued walking than during rest. This means that the rate of removal is much higher in activity than at rest, in agreement with Newman et al (17). The lactic acid eliminated in the sweat and urine (20, 21) and used by the heart for fuel are not enough to explain this large difference.

IV. *Relation between removal of excess lactate and oxygen consumption.* Of eleven experiments in which the subject rested after the ischemia, and ten in which he continued walking, examples are shown in table 2 and in figures 1

and 2, from which it can be inferred: *a*, that the amount of lactate removed from the body per liter of oxygen is much greater during work than during rest (Robinson has found the same thing in experiments quite different in procedure from ours); and *b*, that the rate of removal of lactate does not alter after all of the extra oxygen has been consumed, and the oxygen consumption is once more at its basal level in the resting experiments, or its value for the steady state in the working experiments (figs. 1 and 2). This fact may indicate that removal of lactate and extra oxygen consumption are independent.

The relation among lactic acid production, lactic acid removal and oxygen utilization in muscle has been carefully studied. In isolated muscle lactic

TABLE 2

Relation between oxygen consumption and disappearance of lactate after ischemia of the legs

TIME AFTER RELEASE OF ISCHEMIA	PULMONARY VENTILATION	OXYGEN CONSUMPTION	R.Q.	LACTATE: In blood	LACTATE: Total in body	LACTATE: Amount removed from body calculate from 0 min.	LACTATE: Rate of removal of lactate	OXYGEN DEBT	LACTATE REMOVED PER LITER OF O_2 DEBT
				Subject "Co" resting after ischemia					
minutes	*l./min.*	*l./min.*		*mgm./100 ml.*	*grams*	*grams*	*Velocity constant "K"*	*liters*	*grams*
0	49.2	1.780	1.23	46	36.8*				
2				40	32.0*				
7	14.40	0.496	0.98	30	24.0*				
20	8.30	0.343	0.84	22	17.6*	19.2	0.062	2.460	7.80
				Subject "Co" continuing to walk after ischemia					
0	70.40	3.170	1.05	45.2	36.1*				
1½				41	32.9*				
5				30	24.0*				
7	52.00	2.480	0.87						
10				25	20.0*				
15	53.70	2.470	0.92						
17				18	15.2*	20.9	0.124	1.150	17.4

* Calculated.

acid formed anaerobically can in the presence of oxygen be oxidized or resynthesized into its precursor (5) about one mol of lactic acid being burned to supply energy for resynthesis of 4 mols (15). The rate of resynthesis is to some extent dependent upon the severity of the previous exercise (2). In muscles with intact circulation, the removal of lactic acid is much more rapid than the resynthesis of glycogen (6, 12). In man, Margaria and associates (14) have shown that at most one-tenth of the lactic acid formed during exercise is burned; that up to a considerable rate of work no extra lactate appears in the blood; and that in spite of this, some extra oxygen is consumed in recovery. They called this the alactacid oxygen debt, and concluded that when the excess

oxygen consumption is less than 3 liters, it is not employed in burning lactate. Under the special conditions of our experiments, however, about the same extra oxygen consumption is found, but with a remarkable rise in blood lactate. As shown above, the speed of removal of lactate does not change when all the extra oxygen has been used. All these facts suggest that the removal of lactate is at least partly independent of the extra oxygen used; in other words, that oxygen is not used necessarily in the removal of lactate from the blood stream.

V. *Lactate diffusion from the muscles to the local and general circulation.* In dealing with the dynamics of lactate diffusion from muscle to circulation, we have to consider three fundamental factors: *a*, the absolute amount of lactate accumulated in the muscles; *b*, the speed of the circulation through the tissues where the lactate is accumulated; *c*, the permeability of the muscle cell membrane to lactate.

In order to find out how the lactate produced in the muscles diffuses into the local circulation, the following experiment was employed:

The subject lay on a bed and the circulation of both arms was cut off with sphygmomanometer cuffs at the level of the distal insertion of the deltoids. Then the subject performed exercise with both hands by squeezing rubber bulbs rhythmically at a rate of 60 squeezes per minute. After some time, usually 1 minute 25 seconds, the pressure and the exercise were stopped at the same time and the subject remained resting in bed for 10 minutes. Venous blood was drawn at intervals from an antecubital vein throughout the work and rest periods. The results of some of these experiments are assembled in figure 3. The first part shows how the lactate reaches its maximal value in the local venous blood and the second, the manner in which it decreases.

The data suggest the following interpretations: *A*. At the simultaneous cessation of exercise and ischemia lactate diffuses into the blood stream and decreases promptly after it reaches its maximal concentration. *B*. The shape of the curve in its rise and the early abrupt drop of lactate concentration in local venous blood indicate: *a*, its high diffusibility; *b*, that its production ceases as soon as exercise ceases.

Our results are in many respects similar to those obtained by Hill and collaborators (10) in their experiments "in vitro" with a thin strip of muscle, and with those of Eggleton and Evans (4). Our experiments cannot be compared with those of Flock and Bollman (6) or with those of Sacks and Sacks (19) in which a short period of anaerobiosis in muscles contracting with intact circulation ends with increase of blood flow. In ours, the anaerobiosis is present throughout the whole period of obstruction of the circulation, and the production of lactic acid instead of ceasing after a few seconds of exercise, continues, and is a function of three factors: *a*, muscular mass; *b*, intensity of exercise performed, and *c*, time of arterial obstruction.

SUMMARY

1. Certain interrelations between oxygen consumption, lactic acid production and removal from the blood stream have been studied on normal subjects whose limbs were rendered ischemic while they were walking in a steady state.

2. During the ischemia, ventilation and oxygen consumption were reduced by about 25 per cent.

3. After the ischemia was relieved, ventilation, oxygen consumption and blood lactate all increased far above the control value, and then returned to this level.

4. The process of restoration was much more rapid if the subject continued to walk than if he began to rest when the ischemia was relieved.

5. The amount of extra oxygen used per gram of lactate removed from the body was far greater at rest than during work.

6. No simple relation was found between the rate of repaying the oxygen debt and the rate of removal of lactate. The rate of removal of lactate was the same after the oxygen debt was paid as it was at the beginning of the recovery period.

7. Diffusion of lactic acid from the working muscles to the local venous blood was a rapid process.

Acknowledgments. We wish to thank Drs. Robert Johnson, Robert Darling and William Forbes and the other members of the laboratory for their help in this work.

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THE INFLUENCE OF RICKETS AND OF THE HEALING OF RICKETS ON THE MECHANICAL PROPERTIES OF THE TIBIAE OF RATS[1]

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It is the purpose of this paper to present some results obtained in the study of the influence of a healing diet on the mechanical properties of bones that had been rendered rachitic and then healed for varying periods. Despite the extensive studies on rickets, there is little information about the subsequent influence of rickets on bones after healing. It has been tacitly assumed, once roentgenological healing of rickets could be demonstrated, together with restoration of calcemia, phosphatemia and blood phosphatase, that rickets had left the organism in an optimal physiological state except for any gross skeletal deformities which might remain. Such a conception of rickets is probably too restricted. There is abundant evidence in the literature to support the thesis that rickets is a constitutional disease. Hess (1) has stated that "rickets is a constitutional, metabolic disorder and not simply a derangement of the histological features of the epiphyses. However, it suggests, as a corollary, that there may be a form of rickets associated with but little deformity—a type quite as significant from a metabolic standpoint as that which is characterized by enlargement of the epiphyses, rosary and bony malformation." According to this view, it is possible that the same pathological processes which can cause bony malformations may also initiate subtle and minute pathology in other organs. The recent literature in the field of rickets tends to substantiate this idea.

Therefore, it is entirely conceivable that rickets, clinical or subclinical, might leave residua upon which disease entities are built later in life. The evidence for such a conception has been lacking in the past because of the difficulty of showing objectively that a given disease occurring in adult life could be influenced by or even be a direct result of rickets in childhood. A step in this direction was made by Clark and Mrgudich (2), who investigated the internal structure of bone by means of x-ray diffraction patterns. They observed that healed rachitic bone had lost the preferred longitudinal orientation of the inorganic crystal micelles. This was the first tangible evidence that the healed rachitic bone might be permanently altered, and it raises the question whether such bone can be as efficient mechanically as normal bone. It was in an attempt to answer this question that the present investigations were begun.

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The literature bearing on the solution of this problem is scanty. Gardner (3) has observed that estrogen-treated mice have markedly thickened bones, which suggested to him that these bones might be stronger than those of the controls. He tested this hypothesis by breaking the femurs of mice by a method similar in principle to the one used in these experiments and found that the femurs of estrogen-treated mice were stronger than those of their litter-mate controls.

Experimental methods. Albino rats were drawn from the stock colony at weaning. They were divided approximately equally into nine series, totalling 267 rats, and each series was equally subdivided into litter-mate control and experimental groups. The experimental animals were given the Steenbock-Black rachitogenic diet no. 2965 from 18 to 41 days, the controls being maintained on the stock diet of the colony (Purina Fox Chow). Severe rickets was produced in all experimental animals as evidenced by roentgenological examination. After varying periods on the rachitogenic diet, the animals in the experimental group were given 250 units of calciferol in oil and placed on the stock diet. Prompt roentgenological healing resulted in all cases. After periods of healing ranging from 42 to 275 days, the animals were sacrificed for further study.

TABLE 1

Summary of distribution of experimental animals

NO. OF RATS IN EACH SERIES	AGE ON RACHITIC DIET	DAYS ON RACHITIC DIET	DAYS ON HEALING DIET	AGE KILLED	DIFFERENCE BETWEEN CONTROL AND RACHITIC BREAK, WTS.
I—21	25	20	65	110	Not significant
II—30	34	41	42	117	Significant
III—26	24	19	72	115	Not significant
IV—37	24	18	275	317	Not significant
V—35	23	37	42	102	Significant
VI—33	24	20	72	116	♀ Significant
VII—30	24	20	65	109	♂ Significant
VIII—33	24	41	105	170	Not significant
IX—22	21–24	38–41	124	186	Not significant

Differences in cross-section area between experimental and control animals were significant only for series II and V.

The animals were killed by a blow on the head and the tibiae removed, dissected free of soft tissues and broken while still green. In table 1 is shown a condensed resumé of the treatment of each series.

The apparatus used to determine the breaking weight is a modification of a standard engineering machine for testing structural materials (designed by Prof. H. F. Moore of the College of Engineering). The tibia was supported on two blunt wedges 10 mm. apart, and a blunted knife-edge was placed on the bone from above to apply pressure. The bones were always placed on the wedges in the same manner to insure uniformity. By means of a motor and suitable

reducing gear, a heavy weight was pulled slowly across the appartus so as to secure graded pressure on the bone through the knife edge. A writing point recorded the movement of the weight so that, after proper calibration, the total load on the bone at any point could be calculated. Simultaneously, a record of the deformation of the bone was obtained from a gauge graduated in 0.001 inch, attached to the knife edge. From such data, load-deflection curves could be plotted by the method of triangulation (fig. 1).

These were constructed for about half of the bones. The termination of each curve represents the breaking point. The points YP and YP_1 represent, respectively, the yield points for each bone, when the actual elastic yield occurred. The points on the abscissa each represent one in the series of deflections, which occur on the graph at progressively shorter intervals. When these are spaced equally the curve as reproduced in the figure is then constructed.

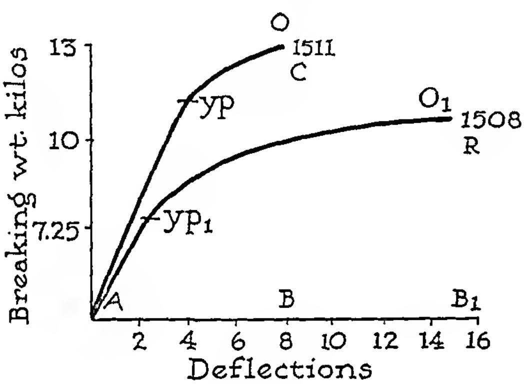

Fig. 1

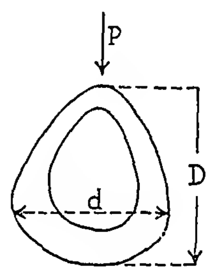

Fig. 2

Fig. 1. Load deflection curves from tibiae. C 1511. Control rat; R 1508. Series III. YP, point of elastic yield or permanent deformation. Areas AOB, AO_1B_1, represent amount of work done in stressing unit volume of material to point of rupture, i.e., measure of toughness of bone.

Fig. 2. Actual outline of tibiae cortex, derived as described in text.

The greater length of the curve from the rachitic bone means that more deflections occurred before breaking. The differences in the deformation of the bones in the breaking process as evidenced by the number of deflections recorded tended to be greater in those series whose healing periods were relatively short, the experimental animals showing greater deformation. The deflection values are significant only when interpreted together with their respective breaking weights.

The areas of the cross-sections of the tibiae near the point of breaking were determined in the following manner: The bones were prepared by grinding the distal fragment of the broken edge of the tibia to a plane perpendicular to its long axis as close as possible to the point of breaking. The marrow cavity was cleaned out with a sharp instrument and filled with chalk, leaving a level solid surface to which was applied a coating of black India ink. The surface chalk was then scraped out of the marrow cavity, leaving a blackened cortex which

stood out in sharp contrast to the white marrow cavity. The bones were then labeled, fixed in petri dishes with paraffin and plaster, and photographed through the bottom, together with a millimeter scale. The negatives were projected against a sheet of drawing paper, giving an enlarged image of the cortex approximately 6 cm. in diameter. The figures were outlined directly in pencil and the markings of the millimeter scale were likewise drawn in. From these figures, measurements of the depth D in the direction of the load P and the horizontal diameter d were made for series II, III and V (fig. 2). The areas of the outlined bone cortices were taken directly from planimeter readings and the results treated statistically. The data on breaking weight and cross-section area were subjected to statistical analysis using Fischer's t factor (4).

In table 2 are shown the condensed data by series on the breaking weight (with standard deviation), number of deflections during the entire time of application of the weight, and the cross section areas of the bones. Both breaking weight and cross section area were significantly lower in the experimental groups in series II, V, the females of series VI, and the males of series VII. In the other groups the trend was usually in the same direction but the differences were not great enough to be of statistical significance. However it is noteworthy that in series IX, the cross section area was greater in the experimental animals; this was true also in the females of series VIII.

The rats in series IX were on a paired-feeding regime, so that both groups were limited to the same calorie intake until one week after return to the stock diet. However, this was not done in series VIII, so it is difficult to account for these findings.

The absence of any consistent variation between the right and left bones is in agreement with the report of Weakley and Dustman (5).

The composite growth curves for series IX are omitted to conserve space but the forms of the curves are identical for both control and experimental animals. While the experimental females never reached the maximum weight of their controls, the experimental males outgrew *their* controls after about the tenth week. And yet the mean cross section area of the experimental groups of both sexes is greater than for the corresponding controls.

That rachitic bone is weaker than normal bone is an established fact. However, no literature was found regarding objective measurements of the strength of healed rachitic bone. To obtain such information has been the objective in this series of experiments.

In the interests of clarity, several of the terms used in this discussion should be defined. By "strength of bone" is meant its ability to resist force; the "elastic strength" being the bone's ability to resist force within its elastic limit; and the "ultimate strength" or "breaking strength" being its utmost ability to resist force, i.e., to resist force to the point of fracture. In this work the ultimate strength has been obtained for all the bones and is the term meant when the word "strength" is referred to. The elastic strength was determined for some of the bones in series II and V.

The fact that series II and V were on a short period of healing, whereas the other series were healed for longer periods may be the chief variable responsible

TABLE 2

Mean values per series or group

SERIES	GROUP	BREAKING WT.	DEFLECTIONS 0.001"	CROSS SECTION AREA
		gms.		*mm²*
I	♂C	12,060	13	2.831 ±.0658
	♂R	11,540	17	2.895 ±.3413
	♀C	10,759	15	2.627 ±.1961
	♀R	10,014	15	2.463 ±.1503
II	♂C	13,359 ±977	13.6	2.263
	♂R	9,345 ±1142	15.1	2.161 ±.2090
	♀C	11,527 ±869	10.7	2.415 ±.2333
	♀R	8,309 ±896	15	2.001 ±.2255
III	♂C	12,951 ±846	14	2.751 ±.3303
	♂R	12,541 ±477	14	2.621 ±.2432
	♀C	10,283 ±1054	13.3	2.210 ±.2116
	♀R	9,795 ±829	14	2.1497 ±.1842
IV	♂C	16,012 ±1765	21	3.9109 ±.4342
	♂R	15,159 ±1336	17	3.3058 ±.1406
	♀C	12,821 ±919	11	2.7787 ±.2071
	♀R	12,291 ±1446	10	2.7781 ±.3239
V	♂C	14,368 ±1301	9	3.2264 ±.3923
	♂R	10,720 ±1110	15.8	2.3555 ±.3135
	♀C	12,539 ±1164	12.6	2.4729 ±.1968
	♀R	9,907 ±624	12.6	2.0668 ±.2305
VI	♂C	15,720 ±865	14	3.8336 ±.2826
	♂R	14,937 ±1363	16	3.5142 ±.2574
	♀C	12,871 ±1270	12	2.8852 ±.3323
	♀R	11,500 ±1137	14	2.6709 ±.1999
VII	♂C	14,927 ±1380	18	3.6929 ±.3071
	♂R	12,106 ±1614	18.7	3.0199 ±.3903
	♀C	12,478 ±1467	16.6	2.6929 ±.1965
	♀R	11,709 ±1680	16.0	2.6516 ±.2503
VIII	♂C	15,861 ±1896	7.3	3.7916 ±.5523
	♂R	16,535 ±2172	7.0	3.4742 ±.5709
	♀C	11,660 ±1089	9.3	2.6525 ±.2509
	♀R	12,655 ±1973	11.0	2.6884 ±.3574
IX	♂C	13,587 ±2897	9	2.4471 ±.5735
	♂R	15,920 ±2071	8.4	3.4059 ±.6436
	♀C	12,231 ±1452	7.7	2.4771 ±.2245
	♀R	11,945 ±1639	6	2.5135 ±.1897

for the significant differences observed in these two series. Since there were no significant differences in breaking weights between the experimental and control animals in series I which had undergone healing for 65 days, the assumption

can be made that somewhere between 42 and 65 days the various factors which entered into the healing of rickets have progressed to the point where they no longer allow for differentiation of healed rachitic bones from those of their litter-mate controls by the breaking strength method herein described. With longer healing periods some of the experimental bones actually showed greater breaking strengths than those of their controls (series VIII and IX). These differences, however, were not significant.

By plotting the breaking weights (load) as ordinates and the deformations (deflection) as the abscissae, a load-deflection curve is obtained (fig. 1) from which can be read the yield point or elastic limit *YP* of the material (the point at which permanent deformity of the bone begins), which is a measure of the elastic strength of the bone. The area *AOB* under the curve is the amount of work done in stressing a unit volume of material to the point of rupture, which is a measure of the toughness of the bone. The experimental bones in series II and V subjected to such an analysis always showed a decreased elastic strength and decreased toughness when compared with similar curves made from the bones of their litter-mate controls.

The question "what are the factors which might affect the breaking strength of bone?" presents itself. On the purely mechanical side, assuming the quality of the bone to be unchanged, the cross-section area, the depth and horizontal diameter of the cross-sections, the configuration of the outlines of the cortex and the density of the bone are all to be considered. As has been mentioned, the areas and diameters have been measured but the evaluation of the configuration of the bone has not been attempted. This property must be known before the data in hand can be assembled for mathematical study to determine from mechanical laws whether the bones in these experiments have been altered in internal structure. If it can be shown that the configurations and the moment of inertia of the experimental bones have not been changed by the treatment of the rats, then the only other tenable explanation is that there has been a change in the fine structure of the bone in those series where significant changes in strength have occurred.

The cross-section areas of the cortices revealed significant differences between experimental and litter-mate controls only in series II and V, the same two series which showed significant differences in breaking strength. Similarly, the depths and horizontal diameters of the experimental bones in series II and V were significantly smaller than those of their controls.

The laws of mechanics state that a diminution in area and in the depth and horizontal diameter result in a diminution of breaking strength, assuming the quality of the material is unchanged. Hence, the bones of the experimental animals in series II and V have been altered by either: a, reducing the strength mechanically with the quality unchanged, or b, that the strength has been reduced mechanically along with some change in the quality of the bone.

In favor of the mechanical explanation is the work of Gardner (3) referred to earlier, who states that the "breaking strength was closely correlated with the amount of bone present as determined by direct observation and by density as demonstrated by x-ray photographs".

The internal structure of the bone (quality) may be altered in several ways: 1. The inorganic crystal micelles may undergo changes in orientation. 2. The inorganic crystal micelles may be altered in shape. 3. The organic matrix may be altered.

Any or all of these might affect the breaking strength of the bones. An alteration in the quality of the bone substance may be arrived at by critical analysis of the configuration of the cross-section of the cortex, but this involves an elaborate graphical and mathematical study of each individual bone from which the moment of inertia must be obtained.

It is obvious then that cross section area is not the sole factor determining the breaking stress and this is confirmed by a curve correlating breaking weight and cross section area, from which it appears that there is not a straight line correlation. There were 352 points used to determine this curve.

Still further, a curve correlating number of deflections and cross section area indicates also that there are quantitative functional factors involved in all three determinations, cross section area, breaking weight and number of deflections, since a straight line graph was not produced.

So far as this one type of test is concerned, it appears that there is no residual defect in the bones of healed rachitic rats if sufficient time is allowed. But this conclusion applies only to resistance to lateral stress. That there are *no* metabolic residua must be determined by other types of examination of bone, such as that described in the accompanying paper.

These experiments further support the growing conception of bone as a dynamically functioning organ participating in many phases of metabolism and therefore affected by many metabolic functions.

We acknowledge with gratitude the continued interest, practical suggestions and technical information supplied by Prof. H. F. Moore of the Engineering Experiment Station.

SUMMARY

1. An apparatus for applying a quantitative breaking test to the bones of rats is described.

2. When tested by this method the tibiae of rats that have been rachitic and then healed, when compared with litter-mate controls showed significant differences in resistance to lateral breaking stress in two out of nine series of experiments involving 267 animals.

3. In one group on a healing diet for 12 weeks there were significant differences in the females, while in another on a healing diet for 9 weeks there were significant differences in the males only.

4. The metabolic significance of the data is discussed.

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AN ATTEMPTED CORRELATION OF MECHANICAL PROPERTIES OF BONE WITH ANTIRACHITIC HEALING AND WITH MOLECULAR STRUCTURE AS DETERMINED BY X-RAY DIFFRACTION TECHNIC[1]

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Numerous studies have been made on the crystal structure of bone by x-ray diffraction technic, with the result that it is generally accepted that the pattern obtained is practically identical with those from various members of the apatite series having the general formula 3 $Ca_3(PO_4)_2 \cdot CaX_2$. Reviews of the earlier work have been given by several investigators (1, 2, 5). Taylor and Sheard (1) found that diffractograms made from powdered apatite (podolite, dahlite, or fluorapatite), dental enamel, normal bone, salivary calculus, tuberculous pulmonary calcification and synthetic tricalcium phosphate showed closely similar crystal structure.

Later workers (2) extended these observations to include a number of additional materials and confirmed the earlier observations in all essential details. In addition data were published on the lattice spacings calculated for as many as 32 lines or rings, also comparative shadow densities. It was concluded that the composition of bone may be represented by the formula $CaCO_3 \cdot n\ Ca_3(PO_4)_2$, where n is not less than 2 nor greater than 3.

While these investigators did not refer to any preferred orientation of the crystals within the osseous structure and did not discuss that possibility, their figure 2, a diffractogram of dental enamel, shows clear evidence of fibering, thus indicating preferred orientation.

Cape and Kitchin (3) published a diaschema of a diffractogram of dental enamel indicating preferred coincident orientation in 9 out of 14 rings represented. The actual picture was not reproduced.

Clark, Bucher and Lorenz (4) published a single diffractogram of normal bone in which there is evidence of preferred orientation in one ring, which corresponds roughly to the position in which such orientation was later found by Clark and Mrgudich (5), although the earlier report did not discuss this feature at all.

Aside from cursory reference, little attention was given in any of these earlier papers to the possibility of altering the molecular arrangement of bone by physiological processes. Bone was regarded as a relatively fixed crystal structure mainly of heterogeneous arrangement.

J. H. Clark (6) was apparently the first to emphasize preferred orientation of patterns obtained from osseous structures, but her observations were confirmed

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in part by several independent investigators (5, 7, 8, 9, 10, 11). There was a progressive trend toward recognition of bone as a mobile structure responsive to physiological and pathological influences, rather than as a fixed crystal structure as first described (1, 2). Thus, Bale (12) concluded that the unit cell dimensions of bone, dentine, enamel, hydroxylapatite and tricalcium phosphate hydrate are identical but that "present diffraction evidence is consistent with a considerable lack of homogeneity in the nature of tooth and bone substance."

There is no very satisfactory agreement on many points reported by different workers which fact has seemed to justify the further investigations reported in this paper. For example, it is not agreed as to the extent of minute alterations in chemical composition of bone under various conditions. Also Bale (12) presented dimensions of unit cell size in bone and apatite that vary considerably from those recorded by Wyckoff (13). These and other facts make it apparent then that the molecular arrangement of bone is still virgin territory and that some of the physiology of bone is dependent upon a correct comprehension of this problem.

Equipment and methods. The x-ray diffraction technic has been in use for about 30 years in the study of crystal structure of metals and the molecular arrangement of rubber, textiles, plastics and other industrial materials. We could thus take advantage of a technic that has been well developed under the exacting conditions imposed by industry.

The theoretical basis for the technic has been explained by Clark (14). The phenomenon of diffraction depends on the lattice patterns in the planes of a crystal. A portion of the x-rays in a beam are diffracted so as to form a funnel-like arrangement which, on a flat film surface at right angle to the axis of the beam, will form a ring. At a constant distance the dimension of each ring will always be constant for a given substance. If the film distance and the radius of a ring are accurately determined these data may be used to calculate, by the Bragg formula, the lattice spacings in Ångström units, characteristic of each plane in a particle. These *d* values become characteristic identifications of certain planes, and a given pattern indicates a specific chemical substance.

If particles are arranged heterogeneously, or without preferred orientation, the rings will be uniform in density and width through the entire circumference. If, however, there is preferred orientation, i.e., particles are arranged in a definite way, then diffracted rays, instead of a funnel-like arrangement, will be grouped so that the ring characteristic of the particular plane involved will show fibering or arcing in that crescentic thickening will appear in one axis, and the ring will be fainter or even broken in the axis at right angle to the first.

Experimental material. The skeletons of the rats used by Schiller, Struck and Reed (15) in a study of the influence of rickets and of antirachitic healing on the mechanical properties of bones had been preserved and were available for use in this study. Since all of the breaking tests had been applied to the midshaft of the tibia it was natural that we selected a contiguous area from which to grind the sections we used. This was a fortunate, if unpremeditated choice, for it was found very early that differences of considerable magnitude

AN ATTEMPTED CORRELATION OF MECHANICAL PROPERTIES OF BONE WITH ANTIRACHITIC HEALING AND WITH MOLECULAR STRUCTURE AS DETERMINED BY X-RAY DIFFRACTION TECHNIC[1]

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Numerous studies have been made on the crystal structure of bone by x-ray diffraction technic, with the result that it is generally accepted that the pattern obtained is practically identical with those from various members of the apatite series having the general formula 3 $Ca_3(PO_4)_2 \cdot CaX_2$. Reviews of the earlier work have been given by several investigators (1, 2, 5). Taylor and Sheard (1) found that diffractograms made from powdered apatite (podolite, dahlite, or fluorapatite), dental enamel, normal bone, salivary calculus, tuberculous pulmonary calcification and synthetic tricalcium phosphate showed closely similar crystal structure.

Later workers (2) extended these observations to include a number of additional materials and confirmed the earlier observations in all essential details. In addition data were published on the lattice spacings calculated for as many as 32 lines or rings, also comparative shadow densities. It was concluded that the composition of bone may be represented by the formula $CaCO_3 \cdot n\ Ca_3(PO_4)_2$, where n is not less than 2 nor greater than 3.

While these investigators did not refer to any preferred orientation of the crystals within the osseous structure and did not discuss that possibility, their figure 2, a diffractogram of dental enamel, shows clear evidence of fibering, thus indicating preferred orientation.

Cape and Kitchin (3) published a diaschema of a diffractogram of dental enamel indicating preferred coincident orientation in 9 out of 14 rings represented. The actual picture was not reproduced.

Clark, Bucher and Lorenz (4) published a single diffractogram of normal bone in which there is evidence of preferred orientation in one ring, which corresponds roughly to the position in which such orientation was later found by Clark and Mrgudich (5), although the earlier report did not discuss this feature at all.

Aside from cursory reference, little attention was given in any of these earlier papers to the possibility of altering the molecular arrangement of bone by physiological processes. Bone was regarded as a relatively fixed crystal structure mainly of heterogeneous arrangement.

J. H. Clark (6) was apparently the first to emphasize preferred orientation of patterns obtained from osseous structures, but her observations were confirmed

[1] Financial support from the Graduate School Research Board, from the Nutrition Research Laboratories and from the Ella Sachs Plotz Foundation is gratefully acknowledged.

in part by several independent investigators (5, 7, 8, 9, 10, 11). There was a progressive trend toward recognition of bone as a mobile structure responsive to physiological and pathological influences, rather than as a fixed crystal structure as first described (1, 2). Thus, Bale (12) concluded that the unit cell dimensions of bone, dentine, enamel, hydroxylapatite and tricalcium phosphate hydrate are identical but that "present diffraction evidence is consistent with a considerable lack of homogeneity in the nature of tooth and bone substance."

There is no very satisfactory agreement on many points reported by different workers which fact has seemed to justify the further investigations reported in this paper. For example, it is not agreed as to the extent of minute alterations in chemical composition of bone under various conditions. Also Bale (12) presented dimensions of unit cell size in bone and apatite that vary considerably from those recorded by Wyckoff (13). These and other facts make it apparent then that the molecular arrangement of bone is still virgin territory and that some of the physiology of bone is dependent upon a correct comprehension of this problem.

Equipment and methods. The x-ray diffraction technic has been in use for about 30 years in the study of crystal structure of metals and the molecular arrangement of rubber, textiles, plastics and other industrial materials. We could thus take advantage of a technic that has been well developed under the exacting conditions imposed by industry.

The theoretical basis for the technic has been explained by Clark (14). The phenomenon of diffraction depends on the lattice patterns in the planes of a crystal. A portion of the x-rays in a beam are diffracted so as to form a funnel-like arrangement which, on a flat film surface at right angle to the axis of the beam, will form a ring. At a constant distance the dimension of each ring will always be constant for a given substance. If the film distance and the radius of a ring are accurately determined these data may be used to calculate, by the Bragg formula, the lattice spacings in Ångström units, characteristic of each plane in a particle. These *d* values become characteristic identifications of certain planes, and a given pattern indicates a specific chemical substance.

If particles are arranged heterogeneously, or without preferred orientation, the rings will be uniform in density and width through the entire circumference. If, however, there is preferred orientation, i.e., particles are arranged in a definite way, then diffracted rays, instead of a funnel-like arrangement, will be grouped so that the ring characteristic of the particular plane involved will show fibering or arcing in that crescentic thickening will appear in one axis, and the ring will be fainter or even broken in the axis at right angle to the first.

Experimental material. The skeletons of the rats used by Schiller, Struck and Reed (15) in a study of the influence of rickets and of antirachitic healing on the mechanical properties of bones had been preserved and were available for use in this study. Since all of the breaking tests had been applied to the midshaft of the tibia it was natural that we selected a contiguous area from which to grind the sections we used. This was a fortunate, if unpremeditated choice, for it was found very early that differences of considerable magnitude

exist among different bones of the same animal, and even among different parts of the same bone. Hence it is necessary only to emphasize that, unless otherwise stated, the data discussed in this paper were obtained from longitudinal slab-like sections. Each section was taken from the outer cortex of a tibia, as nearly as possible in a standard position, at the juncture of the middle and lower thirds of the shaft. At this point the shaft is of least diameter and most nearly cylindrical, and the micelles are more uniformly arranged in parallel.

Most of the bones used for diffraction studies were air dried. Sections ground from fresh bones gave diffractograms identical with those obtained from the

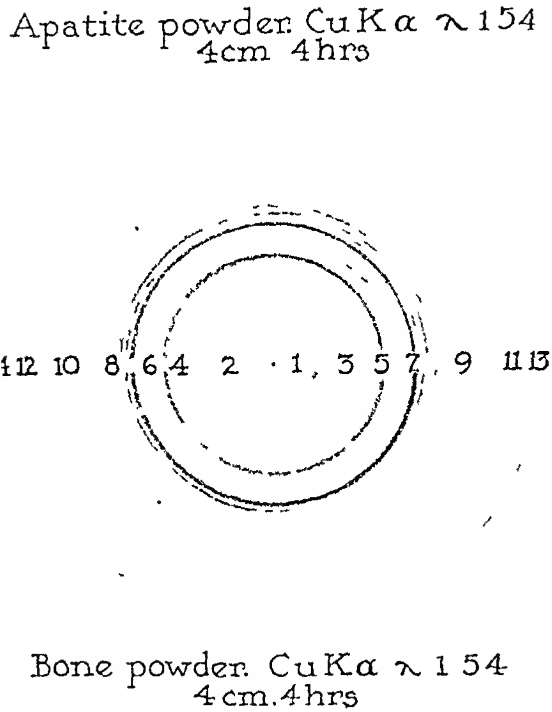

Fig. 1

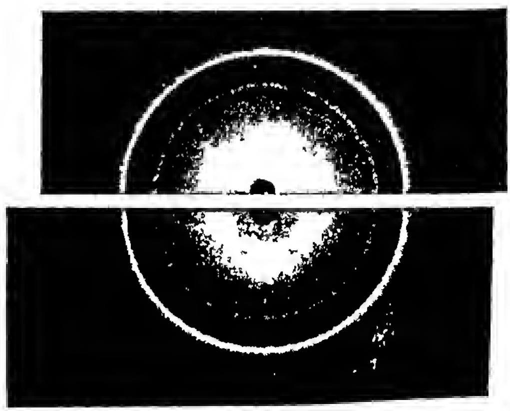

Fig. 1a

Fig. 1a. Actual positive prints of diffractograms from which figure 1 was drawn; same relative positions. Skewed arrangement due to inversion of bone powder picture to eliminate shadow of wire and bead. Many lines visible on original negative are not reproduceable in a positive print.

same material after weeks of drying. On *a priori* grounds there would appear to be no good reason to expect any alteration in crystal structure by the drying process.

Experimental results. More than 800 diffractograms have been made on bones from various sources. For purposes of analysis, patterns were obtained from powdered apatite and from powdered bone obtained by grinding the surface layer of a normal human femur. In figure 1 are semicircular reproductions of these, paired for comparison. The original films are developed as negatives and are difficult to reproduce so all of the fine detail is apparent (fig. 1a). At best, however, a drawing also leaves much to be desired. The original films from

which this figure was drawn are available for examination by anyone interested. It will be apparent, however, that except for minor variations in comparative density in some of the rings, and the presence of one additional ring in the center of the bone pattern, the two are identical. The spacings calculated from the measurements obtained from these patterns are shown in table 1. There is considerable disagreement between these data and those previously published (2, 3, 6, 8, 10, 11, 13), although in the main the discrepancies are not marked. In all subsequent references, rings will be identified by the numbers assigned in this table, and in figure 1, as well as by the *d* values.

In figure 2 are shown diffractograms of bones from animals in series IV as described by Schiller, Struck and Reed (15). That from the control animal is

TABLE 1

RING NO.	APATITE		BONE POWDER	
	Radius	*d*	Radius	*d*
	mm.	*Å.u.*	*mm.*	*Å.u.*
1			4.5	17.11
2	7.5	8.29	7.5	8.29
3	13.0	4.29	13.0	4.92
4	15.75	4.06} 3.89	15.5	4.12} 3.96
	17.25	3.27	17.0	3.80
5	18.75	3.48} 3.43	18.75	3.48} 3.43
	19.5	3.38	19.5	3.38
6	21.25	3.20} 3.12	21.5	3.16} 3.12
	22.5	3.04	22.0	3.08
7	24.25	2.84} 2.78	23.75	2.96} 2.82
	25.5	2.72	26.0	2.68
8	27.5	2.58	27.5	2.58
9	33.0	2.26	33.0	2.26
10	36.0	2.10	36.0	2.10
11	42.0	1.88	42.0	1.88
12	44.5	1.76	44.5	1.76
13	45.5	1.72	45.5	1.72
14	48.0	1.68	48.0	1.68

Film distance 40 mm. Nos. 4, 5, 6, 7 were very broad rings, hence both internal and external circumferences were measured and the *d* values averaged.

typical of about 400 diffractograms from control animals. The most striking thing is the sharp arcing, or fibering, of no. 5 ring. The long axis of the bone is indicated by the line drawn through these arcs. Also there is evidence, less distinct, of fibering in no. 8 ring which coincides in orientation with that in no 5. The preferred orientation displayed here confirms the observations described by others (3, 5, 6, 7, 9). However, it does not confirm other aspects of the pattern described by J. H. Clark (6) in that there does not appear to be any strong orientation on an axis at right angle to that of the micelles of the bone, although there is slight widening of no. 7 opposite the gaps in no. 5.

Further, it is in complete disagreement with the pattern described by Cape and Kitchin (3) in which there was coincident fibering in 9 out of 14 rings.

The pattern, *r* in figure 2, is from an experimental animal of this same series, which was on a rachitogenic diet 18 days and on an antirachitic diet 275 days. The mechanical performance of the bones from this series (IV) as described (15) shows that, so far as the breaking test is concerned, the animals had recovered completely and the bones had regained their resistance to lateral stress.

But when examined by the diffraction technic it is apparent that the characteristic pattern of rickets is still present to a marked degree. There is definite fibering in no. 8, but that in no. 5 is much less distinct than in the control animals. And in both the orientation is in the long axis of the micelles. It must be concluded then that some observations reported previously (3, 6) were not complete. The very dark central band noted by several early workers is very faint in all of our patterns. It can only be concluded, therefore, that some of the features so prominent in many of the earlier diffractograms were due to the imperfections of equipment. At any rate the rachitic state has not produced the marked darkening of the central disk that we recently stressed (16). The

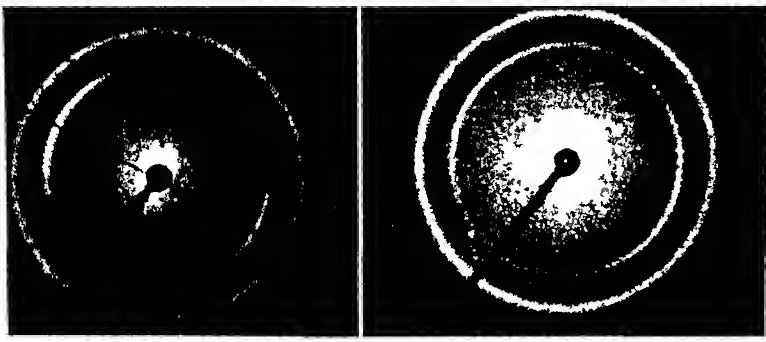

Fig. 2 Fig. 3

1762 c 1766 r

Fig. 2. 1762, Control animal. 1766, Healed rachitic animal. (See text.)
Fig. 3. Positive print from diffractogram film of spicule from buffalo skull. See text.

diffractograms reproduced in that brief report were made with the older equipment now discarded.

The rachitic state, then, produces disorientation in no. 5 ring being extended and the widest part of the shadow becoming less dense. In other bones there was complete coalescence of the arcs at one or both ends, tending to form, or actually forming, a smooth ring like that produced by powdered bone.

The fact that this condition persisted in all but 3 animals out of 37 in this series even after 275 days on an adquate diet raises a question about the significance of this change. The breaking test indicated that the bones of the rachitic animals were quite as strong as those of the control animals of this series. The same was true of all the other series. The degree of disorientation might be expected to show an inverse proportion to the length of time on the healing diet. This has not, however, proven to be the case. If molecular orientation has any physiological significance, it must be in relation to some function not involved in resistance to lateral stress. There are several possibilities, but any discussion of them at this time would be purely speculative.

Since all of the bones used represent terminal stages of individual experiments, as all of the animals in a series were killed at one time, there is no means of knowing whether there was *any* recovery of orientation. To investigate this point an experiment was devised wherein a group of rats was rendered severely rachitic, then half were placed on an adequate diet, and the others received merely a supplement of vitamin D with the rachitogenic diet. At regular intervals one rat was taken from each of these groups and one from a control group. At the present diffractograms indicate that reorientation to the extent of about half has occurred under the vitamin D supplement to the rachitogenic diet while the antirachitic diet has produced considerably less reorientation. This result is so bizarre that it must be repeated for confirmation. It is mentioned

TABLE 2

RING NO.	CONTROL		HEALED RACHITIC	
	Radius	*d*	Radius	*d*
	mm.	*Å.u*	*mm.*	*Å.u.*
1				
2	7.5	8.29	8.25	7.62
3	12.0	5.31		
4	17.5	3.76	18.25	3.62
5	19.0	3.50 } 3 42	19 5	3.55 } 3.40
		3.34	20.5	3.26
6	21.75	3.12 } 3.10	21.75	3.12 } 3.06
	22.0	3.05	22.75	3.01
7	24.25	2 84 } 2.78	25.0	2.78 } 2.73
	25.5	2.72	26.0	2.68
8	27.25	2.60	28.0	2.54
9	32.25	2.37	33.5	2.30
10	38.0	2.08	40.0	2.01
11	41.5	1.92	43.0	1.84
12				
13	46.0	1.71	47.0	1.67
14				

here only to indicate the possibilities of physiological investigation by this technic.

Table 2 is constructed from the data taken from the diffractogram reproduced in figure 2. Comparison with table 1 shows that there are disagreements which may be ascribed in part to the organized gross structure of bone on a protein matrix which blots out certain rings completely while others are modified in a definite way. Since the quantitative differences are more pronounced in the healed rachitic bone, they can scarcely be ascribed to errors in measurement. It seems likely that there are, after all, some chemical differences of small magnitude.

It has been generally assumed that the orientation of crystals in a bone takes place in response to muscular activity. The only test that can be applied to this point is to examine a bone not subject to any muscular activity. After considerable searching it was found that in the supramaxillary sinus of the buf-

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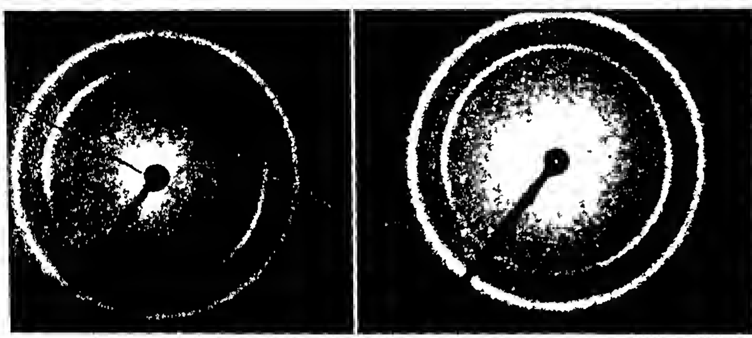

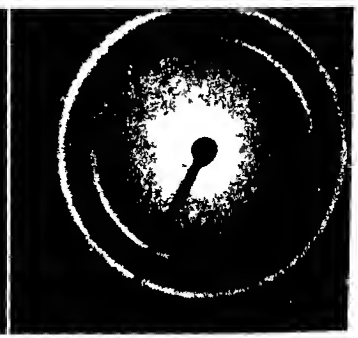

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		3.34		20.5	3.26	
6	21.75	3.12	3.10	21.75	3.12	3.06
	22.0	3.05		22.75	3.01	
7	24.25	2.84	2.78	25.0	2.78	2.73
	25.5	2.72		26.0	2.68	
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falo skull there are rod-like spicules of bone of the same order of dimension as a rat tibia, both ends of which are attached to the same skull bone. Therefore, it may be presumed that these have never been subjeet to muscular aetivity but only to statie stress.

A diffractogram of sueh a bone is reproduced in figure 3. It shows all of the main rings found in the tibia, with nos. 5 and 8 oriented in exactly the same way. Incidentally, this bone had been exposed to weathering for about 60 years.

Subsequently, examination of bones unearthed in areheologieal excavations in Asia Minor, one of which dates to a period 3,000 years ago, the other to about 5,500 years ago, reveals characteristie orientation in both.[2]

Bearing in mind that all of the observations on rickets were made on specimens from what is probably one of the most eompact bones in the rat skeleton, the cortex of the midshaft of the tibia, it is remarkable that the crystal pattern should be so mobile or so responsive to metabolic influence. It is, perhaps, not out of order to mention some data whieh will be reported in detail later that illustrate this point. Such influenees as parathyroid, excess vitamin D, a Ca-deficient diet, strontium or magnesium substitution for ealcium, and estrogens, all produee disorientation of considerable magnitude. Whether these ehanges are reversible has not been determined.

Clark, in a personal eommunieation, has suggested that, instead of grinding sections, a traeer film of paraffin be applied to bones; the orientation of the latter on the micelles ean then be used to determine more aecurately the orientation of the mieelles themselves, sinee paraffin gives finer fibering in a diffraetogram than the bone salts themselves.

SUMMARY AND CONCLUSIONS

1. When examined by x-ray diffraetion technic, the cortices of the tibiae of albino rats show eharacteristie preferred orientation on the long axis of the bones, in eonfirmation of the reports of several earlier investigators.

2. This orientation is most pronounced in the plane having a lattice spacing of 3.43 Å.U. and eorresponds to the 5th ring in the type pattern of powdered apatite.

3. The identity of bone salts with apatite is eonfirmed by the finding of nearly identieal powder diffraetograms of bone and apatite.

4. Disorientation occurs in rickets and appears to be largely irreparable.

5. Disorientation in rickets bears no direct relation to the ability of bones to resist mechanical stress since bones of rachitic rats may be restored but disorientation is not repaired completely in 275 days on an antirachitic diet.

6. The preferred orientation of normal bone is disrupted by many physiological and pathologieal influences, the signifieance of which is being investigated.

7. Whether disorientation has any physiological significance other than as an indicator of a type of metabolic disturbance cannot be determined at present.

[2] One specimen was donated by the Field Museum, through Doetor Von Bonin; the other by Professor Krogman of the University of Chicago.

Our grateful appreciation is extended to Drs. G. L. Clark and S. T. Gross, without whose assistance and advice this investigation would not have been possible; and to Dr. H. C. Struck and Mr. J. K. Ross for the care of the rat colony, thus providing a homogeneous strain of test animals.

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THE EFFECT OF MEAT AND MEAT FRACTIONS ON THE FATTY LIVER OF THE DEPANCREATIZED AND PANCREATIC-DUCT LIGATED DOG

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Fatty infiltration of the liver has been repeatedly observed in depancreatized and pancreatic duct ligated dogs (1–4). That this was not, in the case of the diabetic animal, due to the carbohydrate disturbance was shown by the fact that adequate control of the diabetes with insulin did not prevent the fatty livers (1, 4). The condition was originally prevented by feeding raw pancreas (1, 5, 6) and in recent years by the addition of choline chloride to the diet (4, 7). Enteman and Chaikoff have reported that 35 mgm. of choline chloride per kilogram added to the diet was sufficient for the complete prevention of fatty livers (4).

In almost all studies on the production of fatty livers in diabetic dogs, the source of protein in the diets has been *whole raw lean beef*. Due to the absence of the pancreatic enzymes in depancreatized or pancreatic duct ligated dogs there is an impaired digestion and absorption of protein (2, 11). This leads to a state of protein depletion, which may be further aggravated by the increased urinary excretion of nitrogen that occurs (13). In view of the importance of protein in the prevention of dietary fatty livers (8, 10), it seemed reasonable to consider that the state of protein depletion produced in the depancreatized and pancreatic duct ligated dog might contribute to the production of the fatty livers observed. This impression was confirmed by the results obtained when dried meat powder, which is readily digested and absorbed even in the absence of the pancreatic enzymes, was substituted for raw lean beef in the diet of diabetic dogs.

This report is concerned with the effects of diets containing as a source of protein either extracted meat powder, whole raw lean beef, or meat powder plus meat extract on the fatty livers of depancreatized or pancreatic duct ligated dogs. The meat powder and meat juice were obtained from the Valentine Meat Juice Co., and we are indebted to them for the details of its preparation.[1]

The meat powder contains almost all the protein and fat of the meat. The water extractives which are removed consist of all of the water soluble constituents of muscle, including the salts, carbohydrates, inositol and various nitrogenous bases.

PROCEDURE. Four groups of dogs are reported (table 1). The first group con-

[1] The lean beef is finely ground and extracted in hot water at a temperature just below boiling point in an open steam jacketed kettle. The solution gets enough heat at this point to coagulate most of the coagulable proteins. The liquid portion is passed through a centrifuge and filter presses and concentrated in vacuo to make "meat juice." The solid residue which contains the beef protein is submitted to hydraulic pressure to get all the juice out and then re-extracted with water and finally dried in a steam dryer.

sisted of 15 dogs fed the dried meat powder. Twelve dogs were completely depancreatized and in the remaining 3 the ducts were ligated. Group II consisted of dogs fed lean raw beef; 3 were depancreatized and 3 ligated. The third group consisted of 10 dogs, 7 were given dried meat powder plus the water extractives of meat in the form of Valentine's meat juice, 15 cc. daily. (This was the amount of concentrated meat juice extracted from 300 grams of lean beef.) Six of the

TABLE 1

Groups of dogs studied

NO. OF DOGS	NO. DEPANCREATIZED	NO. DUCT LIGATED	SOURCE OF PROTEIN
15	12	3	Dried meat powder
6	3	3	Raw beef
7	6	1	Meat powder plus meat extract
3	3		Meat powder plus inositol
3	3		Meat powder plus choline chloride

TABLE 2

Diet fed to dogs daily

FOOD	AMOUNT FED PER DAY	CARBOHYDRATE	PROTEIN	FAT	IODINE NUMBER OF FAT
		Per amount fed			
	gms.	*gms.*	*gms.*	*gms.*	
Basal diet					
Skim milk powder	30	17	13.0	0	
Cracker meal	100	81	9.6	4.5	
Dried brewer's yeast	10				
Sugar	30	30			
Salt mixture	3				
Bone ash	4				
Viosterol	2 drop				
Sources of protein					
Meat powder (Valentine)	50	1.5	38	4.5	55
or					
Lean beef	200		40	20	60
Fat					
Cod liver oil	5			5	167
Mazola oil	17			17	120

dogs were fed dried meat powder plus inositol, one of the water extractives of lean beef. In addition, a fourth group of 3 depancreatized dogs were given dried meat powder plus 2 grams of choline chloride daily.

The diet fed the dogs is shown in table 2. The amount of meat powder fed varied from 35 to 50 grams depending on the size of the dog. The nitrogen content of the amounts of meat powder was equivalent to that of 200 to 300 grams

of raw lean beef. The choline content of the meat powder was determined by the reineckate method of Beattie (14). The choline content of the amount of meat powder fed daily was 130 to 200 mgm. On the basis of Chaikoff's report that a dog requires 35 mgm. of added choline chloride per kilogram of body weight to prevent fatty infiltration of the liver, the dogs in this series, with the single exception of dog 15-D weighing 5 kgm., would have required from 300 to 483 mgm. daily to prevent fatty infiltration of the liver. The dog weighing 5 kgm. would have required 175 mgm. daily. The amounts of raw meat fed daily to the dogs on meat contained 150 mgm. of choline.

The dogs were kept in individual metabolism cages and fed twice daily. The depancreatized dogs received insulin daily, in amounts which permitted a mild

TABLE 3

Liver lipids of depancreatized and duct-ligated dogs fed meat powder

DOG NO.	TIME AFTER PAN-CREA-TECTO-MY	WEIGHT		WEIGHT OF LIVER	TOTAL LIPIDS	TOTAL FATTY ACIDS	CHOLESTEROL			PHOS-PHO-LIPIDS	NEU-TRAL FAT	AMOUNT OF MEAT POWDER FED DAILY	CHOLINE CON-TAINED IN MEAT POWDER FED	ESTI-MATED DAILY CHOLINE RE-QUIRE-MENT
		Initial	Final				Total	Free	Ester					
	weeks	*kilo.*	*kilo.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*	*mgm.*	*mgm.*
1-D	6.5	10.3	8.0	358	4.4	3.1	0.27	0.21	0.06	2.95	1.17	35	135	360
2-D	7.5	13.8	6.8	369	5.6	4.4	0.27			2.50	2.80	50	203	483
3-D	10.7	13.3	12.8	395	4.5	3.2	0.23	0.19	0.04	2.66	1.34	35	135	465
4-D	11	13.0	12.3	Sampled	4.6	3.4	0.22	0.19	0.03	2.57	1.78	50	203	455
5-D	12	15.8	10.0	Sampled	10.3	9.0	0.23	0.21	0.02	1.94	8.10	35	135	553
6-L	12	11.8	9.8	267	9.6	8.2	0.32	0.21	0.11	1.98	7.20	35–50	135–203	413
7-D	13	8.0	6.4	232	12.3	10.4	0.33	0.21	0.12	3.29	8.50	35–50	135–203	280
8-D	13	10.3	11.3	403	5.7	4.4	0.24	0.20	0.04	2.72	2.70	50–75	203–270	360
9-L	13.2	13.0	7.4	212	11.9	10.5	0.35	0.22	0.13	1.89	9.60	35	135	355
10-L	13.5	11.8	10.0	273	5.0	3.7	0.29	0.20	0.09	2.64	2.02	35–50	135–203	455
11-D	19	10.3	9.5	480	7.2	5.8	0.24	0.20	0.04	3.04	3.90	50	203	360
12-D	22	10.3	7.8	521	10.8	9.4	0.24			2.12	8.40	50	203	360
13-D	26	11.3	9.2	425	7.9	6.5	0.22			3.00	4.70	50	203	395
14-D	33	9.0	9.2	390	13.1	11.5	0.27			2.04	10.80	50	203	315
15-D	43.4	4.5	5.3	283	5.8	4.6	0.21			2.58	3.10	50	203	175

glycosuria. Urine analyses were done daily and blood sugars at weekly intervals. Liver lipids were done by the methods previously described from this laboratory (15).

In ligating the pancreatic ducts two procedures were used. In dogs 6-L and 10-L in group I, in 16-L in group II, and in 23-L in group III, the ducts were completely ligated and the portion of the pancreas adherent to the gut and containing the ducts was stripped off and removed. The exposed ends of the remaining pieces of pancreas were covered with peritoneum. There was no connection therefore between the portions of pancreas left and the gut. These animals were, of course, partially depancreatized, but none of them developed diabetes. In the other ligated dogs no portion of the pancreas was removed. After ligation

of the ducts, a piece of omentum was inserted between the ducts and the gut to prevent the re-establishment of any connection.

RESULTS. *The results on the 15 dogs fed meat powder are shown in table 3.* The dogs were observed for periods varying from 6.5 to 43 weeks following operation. In 9 dogs the total liver lipids were below 8 grams per cent. In the other 6, the total lipids varied from 9.6 to 13 grams per cent. The other lipid fractions were within normal limits, except for the increase in fatty acids and neutral fat that occurred when the total lipids were elevated. *No fatty infiltration of the liver was observed in the dogs on meat powder alone up to intervals of 11 weeks following operation.* In 2 dogs after 12 weeks the total liver lipids were 10 and 9.6 grams per cent. In 4 dogs after 13 weeks the liver lipids were quite normal in 2 (5 grams per cent) and in the other 2 there was an increase to 12 grams per cent. Five dogs were observed for periods varying from 19 to 43 weeks. The liver lipids

TABLE 4

Liver lipids of depancreatized and duct-ligated dogs fed raw beef

DOG NO.	MEAT FED	TIME AFTER PANCREATECTOMY OR LIGATION	WEIGHT		WEIGHT OF LIVER	TOTAL LIPIDS	TOTAL FATTY ACIDS	CHOLESTEROL			PHOSPHOLIPIDS	NEUTRAL FAT
			Initial	Final				Total	Free	Ester		
	gm.	*weeks*	*kilo.*	*kilo.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
16-L	200	8	11.8	6.8	370	24.2	21.1	0.22			2.44	20.5
4-D*	200	13.6	12.3	12.3	610	14.7	13.1	0.44	0.17	0.260	1.76	12.3
17-L	150	14	17.8	13.5	660	29.9	25.9	0.28	0.15	0.135	2.32	25.5
18-L†	150	15.3	18.3	13.0	385	20.6	17.6	0.30	0.19	0.112	1.47	17.0
19-D	200	21.4	11.0	12.0	471	28.8	27.2	0.31			2.52	26.0
20-D	150	33	23.8	10.0	734	32.0	26.9	0.30	0.19	0.113	1.70	20.2

* Dog given meat powder for 11 weeks. Liver biopsied and analyzed (table 1). Dog then changed to whole meat which she received for 14 weeks.

† Pancreatic ducts ligated and separated from duodenum by omentum.

were normal in 3, and had risen to 10.8 and 13 grams per cent in the other 2. The dog that was observed for 43 weeks had normal liver lipids, 5.8 grams per cent.

The findings in the dogs on meat powder were particularly striking when compared to the results of feeding raw beef. Of 6 dogs (table 4) on raw beef, all developed fatty infiltrations of the liver. The lowest degree was 14.7 grams per cent after 15 weeks, and 4 of the other dogs had more than 24 grams of fat per 100 grams of liver. Dog 4 was particularly interesting because prior to receiving raw lean beef she was fed dried meat powder for 11 weeks. At the end of this time, the liver was sampled and on analysis the total liver lipids were 4.59 grams per cent (table 3). The diet was then changed to raw beef and within 15 weeks the total lipid had risen to 14.7 grams per cent. The other lipid fractions in the livers of the dogs on raw meat showed a sharp increase in neutral fat and fatty acids.

Naturally the results with dried meat powder, contrasted with the results on raw beef, suggested that some component of the extractive fraction of meat was

effective in increasing the deposition of fat in the liver. Therefore, depancreatized and duct ligated dogs were fed 50 grams of meat powder plus 15 cc. of the meat extract. The results are reported in table 5. In 2 of the dogs (nos. 24 and 27) the meat extract was increased to 30 cc. The results obtained with this combination did not result in an increase in the total liver lipids in all the dogs. Three of the dogs (21, 22, 23) developed a pronounced degree of fatty infiltration, and this occurred after intervals of only 5, 7 and 9 weeks following operation. In

TABLE 5

Liver lipids of depancreatized dogs fed meat powder plus meat extract or inositol

DOG NO.	TIME AFTER PANCREATECTOMY	WEIGHT		WEIGHT OF LIVER	TOTAL LIPIDS	TOTAL FATTY ACIDS	CHOLESTEROL			PHOSPHOLIPIDS	NEUTRAL FAT	AMOUNT OF INOSITOL FED DAILY
		Initial	Final				Total	Free	Ester			
Dogs on meat powder and meat extract												
	weeks	*kilo.*	*kilo.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*
21-D	5	9.8	6.8	377	21.4	19.5	0.25	0.19	0.06	1.92	19.2	
22-D	7	9.8	7.0	456	20.2	18.3	0.29	0.18	0.09	2.05	17.8	
23-L	9	9.3	6.8	294	21.5	19.8	0.23	0.15	0.08	1.51	19.7	
24-D	12	12.5	8.3	416	12.50	11.1	0.21			2.04	10.2	
25-D	21.5	10.0	11.0	448	3.94	2.8	0.21			2.73	1.0	
26-D	24	11.8	9.3	550	7.96	6.6	0.22	0.20	0.02	2.73	5.0	
27-D	44	9.8	11.0	450	5.06	3.9	0.24			2.08	2.8	
Dogs on meat powder plus inositol												
28-D	23	12.0	12.8	504	6.2	4.84	0.21	0.19	0.02	2.97	3.0	1
29-D	9.5	9.8	7.3	457	26.6	24.6	0.25	0.18	0.07	1.67	24.6	2
30-D	19	7.8	6.5	298	19.7	18.0	0.26			1.98	17.5	2

TABLE 6

Effect of choline chloride, 2 grams daily, on the liver lipids of depancreatized dogs fed dried meat powder

DOG NO.	TIME AFTER PANCREATECTOMY	WEIGHT		WEIGHT OF LIVER	TOTAL LIPIDS	TOTAL FATTY ACIDS	CHOLESTEROL			PHOSPHOLIPIDS	NEUTRAL FAT
		Initial	Final				Total	Free	Ester		
	weeks	*kilo.*	*kilo.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
31-D	11	10	7.8	371	5.3	2.54	0.246	0.205	0.041	2.9	0.60
32-D	19	9.3	4.8	213	9.4	5.8	0.296	0.258	0.038	4.5	2:90
33-D	26	12.8	11.8	406	7.9	4.1	0.268	0.242	0.026	3.5	1.85

the dogs on meat powder alone, no increase in liver fat occurred until an interval of at least 12 weeks following operation had elapsed. Dog 24 on meat extract, was sacrificed 12 weeks after depancreatization; the liver lipids were 12.5 grams per cent. The other 3 dogs were examined after longer intervals (22–24 weeks) and the liver lipids were normal. The question arose as to whether the lipotropic effect of meat powder might not have succeeded in counterbalancing the effect of the meat extractives as time went on.

Because of the fact that inositol is present in meat extract, 3 dogs were given meat powder plus inositol. *When only 1 gram of inositol was fed daily* for a period of 23 weeks, there was no increase in the total liver lipids. However, *when the dose was increased to 2 grams daily*, 2 dogs developed marked fatty infiltration of the liver, after periods varying from 9.5 to 19 weeks following pancreatectomy.

As the amount of choline in the meat powder was less than would be required to prevent fatty infiltration of the liver, 3 depancreatized dogs were given dried meat powder plus 2 grams of choline chloride daily (table 6). They were kept on this for periods of 11, 19 and 26 weeks which intervals corresponded to the intervals that dogs were kept on meat powder alone. In dogs on meat powder alone, the liver lipids after these intervals were 4.6, 7.2 and 7.9 grams per cent. In the dogs on meat powder plus choline chloride, the liver lipids were 5.3, 9.4 and 7.9 grams per cent. The addition of choline to the meat powder did not influence the total liver lipids.

Discussion. In the depancreatized and duct ligated dogs fed raw beef severe fatty infiltration of the liver occurred within 8 weeks after operation and the increase in liver fat was present throughout the experimental period, as shown by analyses done after 8, 14, 15, 21 and 33 weeks. The livers of the dogs fed dried meat powder and analyzed at corresponding intervals showed no comparable fatty infiltration. In the livers of the dogs fed dried meat powder plus all the extractives there occurred an initial increase in fatty infiltration which did not persist beyond the 15th week. However, when inositol, one of the extractives, was fed there was a marked increase in liver fat after intervals of 9 and 19 weeks. The fact that fatty infiltration of the liver did not persist in the dogs fed the meat powder plus all the extractives suggests that the lipotropic effect of the meat powder counteracted the effect of the extractives when fed in the form of meat juice. These results demonstrate the lipotropic effect of meat powder and conversely the "lipogenic" effect of raw meat in dogs deprived of the external pancreatic secretion. The experiments also indicate that the effect of raw meat is due to some substance or substances contained in the extractives of meat. As regards the fat promoting possibilities of the water extractives of meat, Marks and Young (16) observed that in dogs rendered permanently diabetic by injections of an extract of the anterior pituitary gland, ketosis did not occur if the animals were fed meat which had been extracted with hot water. When raw whole meat was fed, ketosis developed. The investigators mention that "the promoter of ketogenesis is not protein per se, but some water soluble constituent of muscle tissue." They do not mention the condition of the livers of the animals but it is interesting that in their dogs raw meat led to an increased excretion of ketone bodies which suggests that the rate of production of ketone bodies by the liver was increased.

The administration of choline has been one of the methods of preventing fatty infiltration of the liver. In depancreatized dogs 35 mgm. of choline chloride per kgm. added to the diet daily was sufficient to prevent fatty livers. As we have pointed out, the amounts of choline contained in the meat powder fed to the dogs in this study would not have been sufficient to prevent the deposition of

fat in the liver (table 3). Furthermore, *the choline was not added to the diet in the form of choline chloride* but was only that which the dried meat powder contained. This is important because raw lean beef also contains choline, about 75 mgm. per 100 grams. The dogs on raw lean beef (table 4) received about 150 mgm. of choline in the 200 grams of beef fed, yet all these dogs developed fatty livers. Depancreatized dogs reported by Chaikoff received 500 grams of lean beef daily, which would contain 340 mgm. choline, and yet *unless choline* was added as such the animals all developed fatty livers. The absence of any appreciable degree of fat deposition in the livers of depancreatized dogs fed dried meat powder suggests that the lipotropic effect is independent of the choline content.

The question then arises as to why meat powder is effective in controlling fatty infiltration of the liver in depancreatized and duct ligated dogs. We feel that the lipotropic effect of meat powder is based on the fact that the protein body stores of depancreatized dogs become depleted. Dried meat powder is more readily absorbed and digested than raw lean beef, thus making available to the animal the protein ingested. Although excretion studies were not done in this group of dogs, the daily amount and state of the feces were observed, and the amounts were not increased, as is the case in the depancreatized and duct ligated dog fed raw beef (2). The fatty liver of the depancreatized dog is therefore actually similar to the dietary fatty liver that results from low protein diets. When protein is given in a form that is more readily absorbed, despite the lack of the pancreatic juice, the state of protein depletion is counteracted and fatty infiltration of the liver does not tend to occur.

The fact that lipotropic substances such as choline prevent fatty infiltration of the liver in depancreatized dogs on raw beef diets may be due to the fact that choline stimulates the phospholipid turnover in the liver and thus counteracts the effect of the extractives of meat contained in whole raw beef. The rôle of raw pancreas, pancreatic juice, or pancreatic extracts in preventing fatty infiltration of the liver in the depancreatized dog is to increase and facilitate the digestion and absorption of the protein in the diet and thus provide the liver with an adequate amount of protein.

SUMMARY

Twenty-six depancreatized and 7 dogs deprived of the external secretion of the pancreas were studied for periods varying from 6.5 and to 43 weeks after operation.

In 15 dogs the source of protein in the diet was dried meat powder, from which the water extractives of the meat had been removed. In 6 dogs protein was given as raw lean beef. Seven dogs received meat powder plus the water extractives of meat. Three dogs received the meat powder plus inositol, which is present in the water extractives of meat. Three dogs were given the meat powder plus choline chloride daily.

None of the 15 dogs on meat powder showed any very pronounced degree of fatty infiltration of the liver. The highest total liver lipid was 13 grams per cent. In 9 of the dogs the total liver lipids were below 7.9 grams per cent.

All of the dogs on raw meat developed pronounced fatty infiltration of the liver. This occurred within 8 weeks after operation.

Of 7 dogs fed meat powder plus all of the water extractives of meat in the form of meat juice 3 developed fatty infiltration of the liver of 20 grams per cent within 5 to 9 weeks. In another dog the liver lipids were 12.5 grams per cent after 12 weeks. The other 3 dogs were examined after 22 and 24 weeks and the liver lipids were normal. Of 3 dogs given meat powder plus inositol, the 2 that received 2 grams of inositol daily developed severe fatty infiltration of the liver.

The addition of choline chloride in dogs receiving dried meat powder did not have any influence on the total liver lipids.

It is suggested that two factors contribute to the fatty liver of the depancreatized and pancreatic duct ligated dog. First—a state of protein depletion brought about by the impaired digestion and absorption of protein occurring in such animals. Second—the presence in meat of some substance or substances which are capable of producing fatty infiltration of the liver.

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THE ACTION OF ELECTRICAL STIMULI ON THE TURTLE'S VENTRICLE

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The observation that the latency of the ventricular responses to brief threshold electric pulses may be long (over 100 msec.) suggested that this tissue was an ideal object for the study of the events which take place during the stimulus-response delay. Knowledge of those events should give insight into the problem of the mode of action of electrical stimuli on excitable tissues.

METHOD. The turtles were pithed. The heart was then excised and a quiescent ventricle was obtained by removal of the atrium. The ventricle was laid on filter paper moist with Ringer and was fixed to a board by 3 or 4 pins. After placement of 3 to 7 electrodes the preparation was covered by a large glass funnel, thus ensuring a relatively closed moist chamber.

The electrodes were usually chlorided silver needles attached to light flexible copper wire. They were supported by plasticine blocks and adjusted so that the tips rested lightly on the external ventricular surface. Although the muscle was fixed, some movement of the electrodes was unavoidable during the ventricular contractions. This movement led to shifts of contact which resulted in artifacts in the electrical records. Thus, in figure 2, the sharp downward swing that indicates the arrival of the impulse to the proximal recording leads (see fig. 1A), is preceded by a gradual rise, which is interpreted as a movement artifact. This interpretation is based on two considerations. First, the excursion attributed to movement was always larger for the leads more distant than for those closer to the stimulated region. Second, when records were taken with the stimulating cathode as one of the leads (fig. 1C), there was never seen any initial positivity preceding the negative excursion (figs. 3, 5, 8 and 9).

The electric responses of the ventricles were sometimes recorded from a cathode-ray oscillograph, after 5 stages of direct-coupled amplification. The amplifier transmitted the electric events without distortion. More commonly, the responses were registered by means of 4 Grass ink-writing galvanometers and their associated resistance-capacity coupled amplifiers. A response could thus be recorded independently from 4 different regions in the muscle (see fig. 1A). Multiple recording, from several regions, was of advantage for the measurement of conduction velocity and for the determination of the site of origin of a stimulated impulse.

The input to the amplifiers was on push-pull. The ventricle was usually grounded through one of the fixing pins. When the responses were recorded

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at the stimulating cathode—i.e., when an electrode was used both as the cathode and as a recording lead—the ground connection was from the middle tap of a

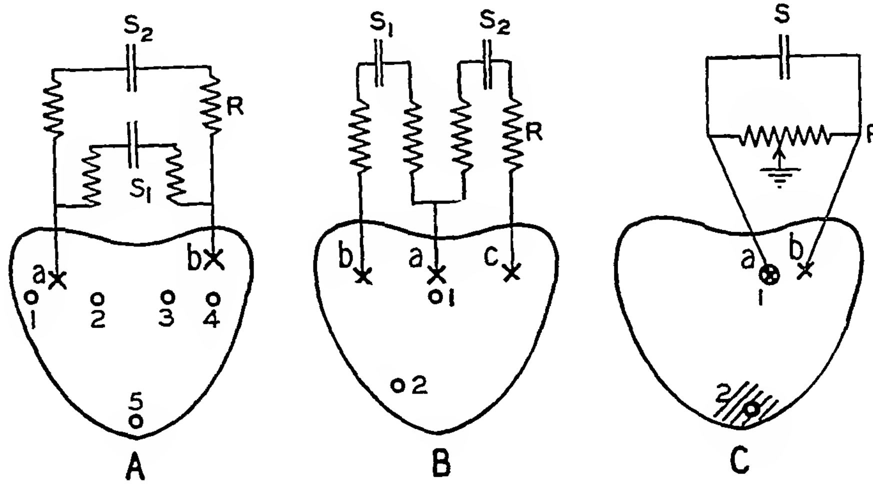

Fig. 1. Diagrams of some of the electrode arrangements used. The crosses (*a* to *c*) represent stimulating electrodes, the circles (1 to 5) leads for recording. S_1 and S_2 stand for the two brief condenser discharges. *R* denotes resistances of 10,000 to 100,000 ω. *P* is a 5,000 ω potentiometer with the middle tap grounded. The hatching at the bottom of C indicates a crushed region. Other points were often crushed, e.g., the regions in contact with the stimulating electrodes *b* and *c* in B, and also those at *b* in both A and C. Additional leads were often included in arrangement B. The records in A were usually from *1*, *2*, *3* and *4*, to *5*, respectively.

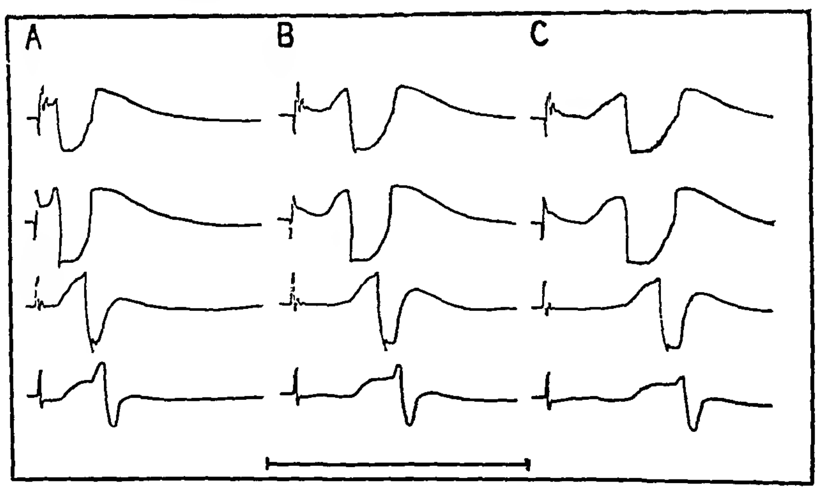

Fig. 2. Increase of stimulus-response delay shown by multiple recording. Electrode arrangement as in figure 1A. Responses to single brief stimuli. Intensity of the shocks (in conventional units): A, 35; B, 30, and C, 29. The first diphasic excursion in the records is the stimulus artifact. The beginning of the response is indicated by the sharp downward swing. The upward excursion which precedes this swing is a movement artifact. Time calibration: 1 sec. The latencies of the responses are tabulated in table 1.

This and other multiple records were taken with ink-writing galvanometers. Only the beginning of the responses is usually reproduced. The later "T" wave is omitted because unnecessary for present purposes.

5,000 ω potentiometer which shunted the stimulating electrodes (fig. 1C). Adjustment of this potentiometer minimized and balanced the stimulus artifacts.

Single records with the cathode-ray oscillograph were sometimes obtained from leads on uninjured tissue (see fig. 1B). More often, however, those records were led from an uninjured to a crushed region of the ventricle (fig. 1C). Multiple recording was usually made with all the leads on uninjured tissue (fig. 1A).

The stimuli were condenser discharges, usually with a time constant of about 0.8 msec. In 1 msec. the shocks had subsided to less than 20 per cent of the initial voltage; and in 2 msec. the discharge was for practical purposes entirely over. Two thyratron controlled stimulators were used. They were tripped by an additional condenser discharge of variable time constant. One of the stimulators was tripped directly, the other after a delay determined by a variable shunt capacity. Two independent shocks with variable time interval (1 to 2,000 msec.) could thus be delivered either through separate or through common electrodes. Resistances of 20,000 to 100,000 ω in series with the output of each stimulator ensured that independence when the two shocks were delivered to a single pair of electrodes. The condensers were always charged to 90 v. The stimuli were controlled by a lineal output potentiometer, which fractionated that voltage. Because of the series resistances the potential drop at the ventricles was much less than the output. The strength of the shocks is given as peak output volts—i.e., as conventional units for the tissue. When repetitive stimuli were applied the pulses were rectangular, with variable amplitude and duration, and with frequency controlled by an oscillator.

The stimuli were usually delivered through two electrodes placed on uninjured tissue (see fig. 1). Often, however, one of the stimulating electrodes was on a crushed region of the ventricle. The purpose of this procedure was to ensure that the impulses should arise at the undamaged region in contact with the other electrode. Tests made with the arrangement schematized in figure 1A, but with the stimulating electrode *b* on crushed tissue, indicated that the impulses always started at *a*, regardless of the polarity of the shocks.

RESULTS. A. *The stimulus-response delay.* The latency of a response recorded at some distance from the stimulated region depends on the duration of three different processes: the utilization period of the stimulus, the stimulus-response delay at the site of origin of the impulse, and the conduction time from this site to the recording point.

The utilization time can be made negligibly small by using sufficiently brief shocks. This is especially true in the heart, where the other time intervals are long. The duration of the shocks used in the present experiments (time constant 0.8 msec.) may be neglected for the computation of the stimulus-response delay.

The conduction time may be eliminated by recording the responses with one lead at the site of origin. In many observations this procedure was adopted (fig. 1C). It is possible, however, that an impulse might not necessarily always start at the cathode, but in some unknown neighboring point. An undetermi-

nable time for conduction would then vitiate the measurement of the stimulus-response delay. This possibility was explored by recording the response from several leads in different regions of the muscle. The stimulating cathode was placed between two of the leads (fig. 1A). A shift of the site of origin of the responses toward either of those two leads would result in an increase of the latency at one of them, but also in a decrease of latency at the other. A lengthening of the delay of the responses, on the other hand, would cause a similar increase of the latency at both leads (fig. 2, table 1). Possible changes of conduction velocity were tested by measurements of that velocity from the records at other leads properly aligned with respect to the stimulated point.

Only rarely was there any evidence that impulses had started at points relatively distant from the cathode. In those cases the latency at one of the leads showed a sudden change which was not paralleled in the records from other regions of the ventricle. In the great majority of the experiments, however, except for the observations which will be described later under the heading "anodal stimulation," any given change of latency was approximately the same

TABLE 1

Latencies (in msec.; error ±2) of the responses reproduced in figure 2

The numbers 1 to 4 refer to the tracings in the figure, from above downwards; the letters A to C refer to the corresponding stimuli. The first 4 columns give the actual latencies; the columns 2–1, 3–2 and 4–3 indicate the differences of latency from one to the following recording point in the ventricle.

	1	2	3	4	2–1	3–2	4–3
A....	80	100	200	265	20	100	65
B....................	220	240	340	415	20	100	75
C....................	305	325	450	535	20	125	85

at all the multiple leads. It is inferred, therefore, that those changes were not due to modified conduction velocity or to a shift of the point of origin of the responses, but were due to variability of the stimulus-response delay. It is inferred also that the impulses started usually precisely at the stimulating cathode.

The responses to strong or long shocks had a shorter delay than those to weak or brief stimuli. Figure 2 illustrates the influence of the voltage on the delay of responses recorded with multiple leads. The same phenomenon is illustrated in figure 3 for responses recorded at the stimulating cathode. The latencies corresponding to two series of observations with variable stimuli are plotted in figure 4, together with the conduction velocity of the impulses. Whether the strength of shocks of constant duration (A) or the duration of equally intense shocks (B) was changed, the latent period of the responses was brief and relatively constant as long as the shocks were well above threshold. As the shocks approached either threshold strength or threshold duration, the latency increased markedly.

In some ventricles just-threshold discharges were applied with various time-

constants and the corresponding voltages. Only random, but no systematic changes of delay were seen when the time-constant was systematically varied in either direction. The inference is supported, therefore, that the significant feature of the stimuli which determines the delay is the amount, in time or in strength, by which the shock exceeds threshold.

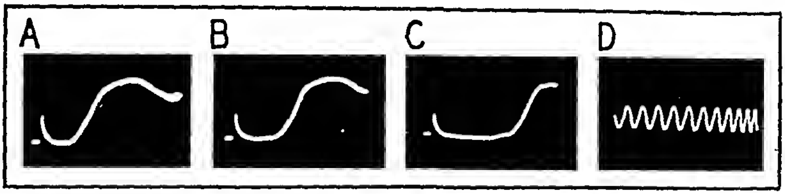

Fig. 3. Variable latency of responses recorded at the stimulating cathode. Electrode arrangement as in figure 1C. The break at the beginning of the records denotes the time of application of a cathodal stimulus. A, B and C show the responses to shocks of diminishing strength. D, time calibration, 100 cycles.

This and other records of this type were photographed from a cathode-ray oscillograph with direct-coupled amplification. Upward excursions denote negativity of the lead on intact tissue with respect to that on a crushed region.

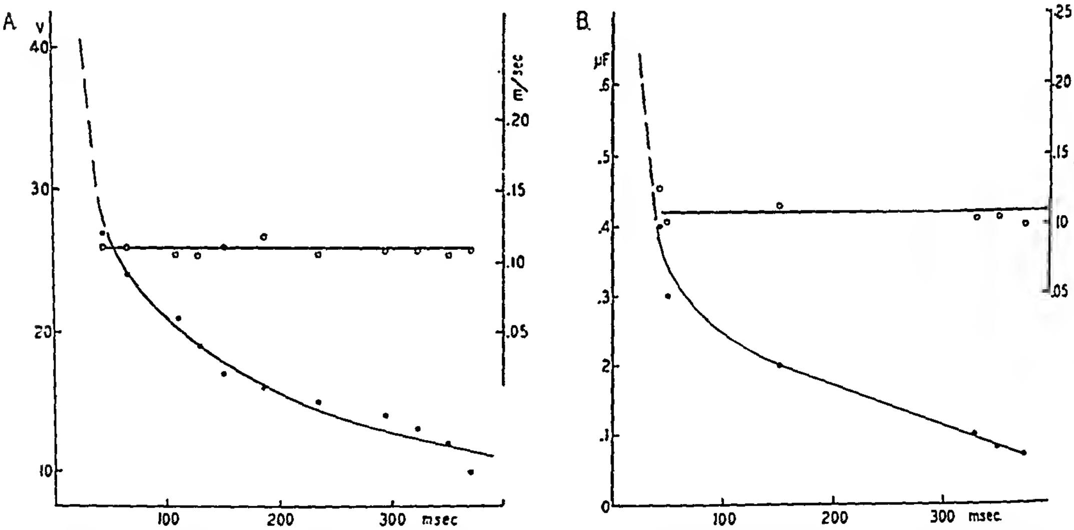

Fig. 4. Changes of latency as a function of the strength (A) or duration (B) of the stimuli. Electrode arrangement as in figure 1A. The latencies plotted (abscissae; dots; msec.) were those at lead *2* in the diagram. The conduction velocities (circles) were measured from the records from leads *3* and *4*; the conduction for each response is plotted at the abscissa corresponding to the latency of that response at *2*. The left scale of ordinates in A is the variable voltage of a condenser discharge with constant capacity (0.3 μF); the left scale in B is the variable capacity of a discharge of constant voltage (20). The right scales of ordinates are for the conduction velocities. The extrapolation of the curves at the left (broken lines) is based on the results of other similar experiments.

Pronounced systematic changes of delay could take place in the successive responses to a series of constant, slightly suprathreshold shocks delivered at a steady rate. Whether this change was an increase or a decrease depended on the rate of stimulation. Stimuli slower than 1 per 10 sec. resulted usually in a progressive decrease of latency, if the strength was such that the first shock

was just above threshold—i.e., if the delay was initially long. A progressive increase of delay, on the other hand, was usual when the shocks were applied at rates faster than 1 per 5 sec. The phenomenon is illustrated in figure 5. With these relatively fast rates, if the first shock in the series was only slightly above threshold, only a few responses were usually attainable, the heart ceasing soon to respond to the constant stimuli.

A description of the changes of excitability of the turtle ventricle after repeated activity is necessary for the understanding of the changes of delay which occur in the course of repetitive activation. In addition to the well-known excitability cycle corresponding to a single response, there are other cumulative variations of excitability which take place after repeated activity. Thus, the threshold of the tissue was usually found lower after repeated responses at a regular, relatively slow rate, so that in the course of a series progressively

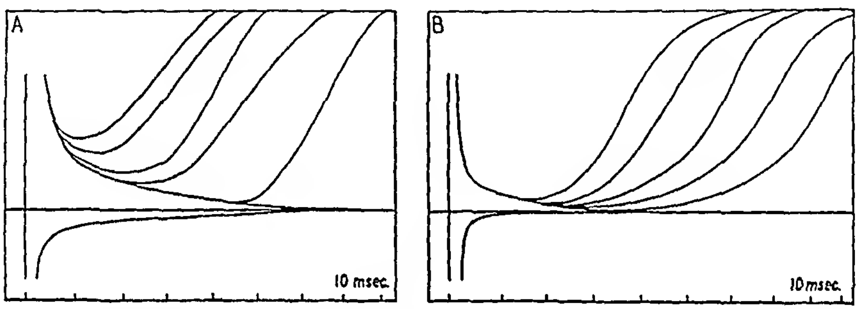

Fig. 5. Increasing latency of successive responses to constant stimuli. Electrodes as in figure 1C. The tracings were drawn from the original records by projection through an enlarger. The horizontal line toward the lower third of the figures is the resting potential line. Upward excursions denote negativity of the electrode common to the recording and stimulating circuits. The upward tracings correspond to series of cathodal shocks delivered at intervals of about 3 sec. in A and 5 sec. in B. A series of responses with increasing latency ensued; the sixth shock was ineffective. The downward excursion in both A and B corresponds to an anodal shock of the same intensity as the corresponding cathodal stimuli. In A the shocks were relatively strong; the first response has accordingly a brief latency. In B, from another ventricle, the shocks were only slightly above threshold, hence the long latencies as compared with A.

weaker shocks could cause activity. That the change was cumulative is indicated by the fact that a given weak stimulus could be effective after many responses (e.g., 10 to 15), whereas it was subthreshold when tested after only a few contractions (e.g., 4 to 6).

As opposed to the increase of excitability commonly seen in a series of responses at a slow rate, a decrease of excitability was the rule when the responses were elicited with a relatively fast frequency. Thus, in order to obtain a series of beats at 5-sec. intervals (or less), if the first stimulus was just-threshold the shocks had to be progressively intensified.

The changes of stimulus-response delay seen in a series of responses can be attributed, therefore, to changes in excitability. Or rather, in order to analogize these changes with those in figures 2, 3 and 4, the statement can again be made that, when in the course of a train of responses the constant shocks become

increasingly closer to threshold, the delay increases; and conversely, when the shocks become increasingly suprathreshold, the delay decreases.

B. *The electrical signs of the events at the stimulated region during the stimulus-response delay.* These electrical signs were studied in records obtained with the electrode arrangement shown in figure 1C. The region of the ventricle in contact with the stimulating electrode *b* was usually crushed. Shocks were delivered with the cathode at *a* (cathodal shocks) or with the anode at that electrode (anodal shocks).

Typical results are illustrated in figures 3 and 5. The part of the tracings which preceded the propagated response, when it was present, showed an asymmetry in the records obtained with cathodal, when compared to those obtained with equal anodal shocks. The negativity of the common electrode in the cathodal records usually, although not invariably, was larger and more prolonged than was the corresponding positivity in the anodal records (fig. 5). Such inequality could be due to an asymmetry in the electrodes and amplifier. To test for this possible source of error some records were taken in similar conditions but with the electrodes lying on filter paper moist with Ringer, instead of on a ventricle. These tests yielded quite symmetrical tracings. It may be inferred, therefore, that the inequality in the records from the ventricle was due to a phenomenon which occurred at the tissue.

The asymmetry was more marked for stronger than for weaker subthreshold shocks. When just-subthreshold cathodal shocks were repeated at intervals of 0.5 to 3 per sec. the residual negativity tended to decrease. There was only a slight or no difference in this negativity when a comparison was made of just-subthreshold with just-threshold shocks.

The negativity after threshold cathodal shocks gradually declined until the beginning of the propagated response. When a series of responses with progressively longer delay was obtained by repeated application of a constant shock, the negativity which preceded the spikes could be quite similar for all the records. As shown in figure 5 the responses then merely started progressively later along the curve which obtained when the shock became subthreshold. Indeed, the responses with a long delay (over 50 msec.) could start at the resting potential level.

C. *Summation of subliminal shocks.* In a series of observations a first cathodal shock of a fixed subthreshold intensity (about 90 per cent) was followed at various time intervals by a second shock of the same polarity. The strength of the second shock which would just stimulate was measured. A summation of excitatory effects was present up to intervals of about 20 msec. (10 to 45 msec. in different preparations)—up to that time the second shock stimulated with intensities which would have been subliminal had it not been preceded by the first one. Beyond that time interval a depressed, instead of an enhanced excitability was seen. With only minor quantitative differences these results are confirmatory of those reported by Gilson and Peugnet (1932).

In another series of observations fixed brief time intervals were selected and the strength of a threshold second shock was measured when the intensity of the

first shock was varied in the subthreshold range. Typical results are shown in figure 6 for 3 time intervals in a single preparation. It is clear that even quite subthreshold shocks (20 per cent) leave a remnant of excitation for as long as 7 msec., so that the effeets of a seeond test shoek are facilitated.

Summation of two subliminal shoeks could be obtained not only when the two stimuli were applied through the same pair of electrodes but also when the shocks were delivered through a common eathode and two independent anodes (fig. 1B). The results were similar with either arrangement of the eleetrodes.

In a third series of observations trains of repetitive stimuli, with constant brief individual duration of the pulses, were delivered through a pair of electrodes. The frequency of repetition—i.e., the interval between the pulses—was varied and the threshold intensity was measured and eompared to that of a single pulse. A summation of subliminal effects was seen only when the interval separating the pulses was less than the critical interval (about 20 msec.) revealed by the two-shock experiments described above. With frequeneies slower than that corresponding to this interval, no response took place unless the first shock succeeded in stimulating.

The summation with short intervals could be cumulative. Thus, a train of 20 or more pulses could be effective when a train of only 5 to 10 stimuli failed, the other characteristics of the shocks remaining constant.

D. *The interaction of two shocks of opposite polarity.* As already stated, the different latencies of the records from different points in the ventricle could indicate the site of origin of a response (p. 53). The terms cathodal and anodal will be used in this section to designate the polarity of the shocks with respect to the electrode at which responses originated.

Whether a threshold cathodal shock was preceded or followed by an anodal pulse, stimulation could be prevented—i.e., the response to the cathodal shock could be cancelled—if the interval between the two shocks and the intensity of the anodal pulse were appropriate.

In figure 7 are shown the results of a typical experiment. The cathodal shocks had a peak voltage of 110 per cent of threshold. Quite weak anodal pulses, either preceding or following the cathodal, sufficed to cancel the responses if the interval between the shocks was brief. As this interval was prolonged the anodal shocks had to be stronger for cancellation. Finally, if the interval was still longer, the strongest anodal shocks which the stimulator could deliver were incapable of preventing the responses. The results obtained from an anodal following a eathodal shock confirm similar observations made by Gilson and Peugnet (1932). The effects seen with anodal shocks preceding a cathodal stimulus extend the somewhat different test of the same problem made by these authors. In agreement with them the excitability of a stimulated region was found first deereased but later, after about 20 msec., increased after application of an anodal shock of moderate strength.

The two branehes of the curve in figure 7 are not symmetrical. As is the case in that figure, the interval within which the stimulating ability of a cathodal shock eould be canceled was usually briefer if the anodal shock preceded than

if it followed the cathodal. The span of this interval varied also with the strength of the cathodal shock employed. A comparison of the curves obtained first with a just-threshold, and later with a well-suprathreshold (e.g., 130 per cent) cathodal stimulus, showed that not only did stronger anodal shocks have to be used at any given interval for cancellation of the response to the greater stimulus, but that the intervals within which cancellation could be obtained were shorter.

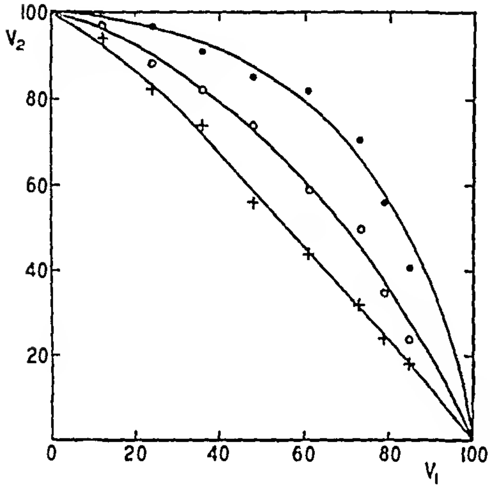

Fig. 6

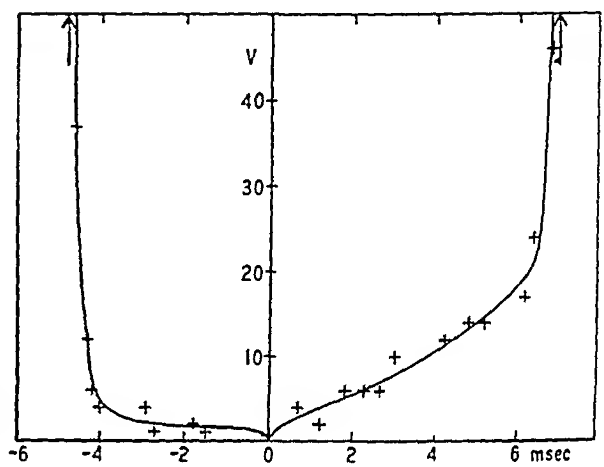

Fig. 7

Fig. 6. Summation of two subthreshold brief shocks at fixed interval. The shocks were of the same polarity and were applied through one pair of electrodes. Abscissae: intensity of 1st shock as per cent of the threshold when delivered alone. Ordinates: threshold intensity of the 2nd shock in a scale similar to that of the abscissae. Lower curve (crosses): interval between the shocks 2.2 msec. Middle curve (circles); interval 3.2 msec. Upper curve (dots): interval 7.4 msec. The observations were made on a single ventricle. The thresholds for each shock were measured at the beginning and end of each series; they remained practically constant.

Fig. 7. Cancellation of the response to a threshold cathodal stimulus by an anodal pulse. Ordinates: intensity (in conventional units) of the anodal shock sufficient for cancellation. Abscissae: interval in msec. between the two shocks; positive values indicate that the anodal shock followed, while negative values mean that it preceded the cathodal stimulus. The two arrows at the top indicate that at the corresponding intervals the strongest anodal shock delivered by the stimulator (about 80 in the scale of voltages used) failed to cancel the responses. The latency of the responses was about 30 msec. when no anodal pulses were applied.

Emphasis is placed on the fact that the critical interval beyond which a following anodal shock did not cancel the response to a preceding cathodal stimulus could be briefer than the stimulus-response delay. Indeed, when just-threshold cathodal shocks were employed the critical interval for cancellation (usually about 8 msec.) was much shorter than the delay of the response (usually about 50 msec.). This difference is strikingly illustrated in figure 8. A strong anodal shock applied 9 msec. after a cathodal stimulus not only failed to cancel the response, but actually shortened its delay.

Were the critical interval for cancellation always equal to the stimulus-response delay, then only the banal conclusion could be drawn that an anodal shock fails to stop a response after it is initiated. But, since the delay may be much longer than the cancellation interval the more interesting inference is reached that there can be a relatively long period after the application of a threshold stimulus and before the initiation of the propagated response, during which strong anodal shocks cannot prevent the appearance of the response.

In some observations a fixed brief interval (e.g., 3 msec.) was selected between the two opposite shocks. The strength of the cathodal stimulus was varied, and the voltage of the anodal shock was determined which would cancel the response. A non-lineal relationship appeared, that is, if the intensity of the

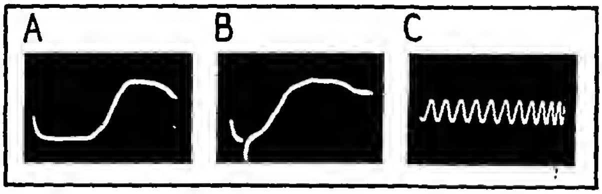

Fig. 8. Lack of cancellation of the response to a threshold cathodal stimulus by a strong anodal shock applied 9 msec. later. Electrode arrangement as in figure 1C except that the stimulating electrode *b* was on crushed tissue.

A. Response at the cathode to a threshold cathodal stimulus applied alone.

B. The same cathodal stimulus followed by an anodal shock 5 times stronger. This anodal shock did not produce any response when applied alone.

C. 100 cycles.

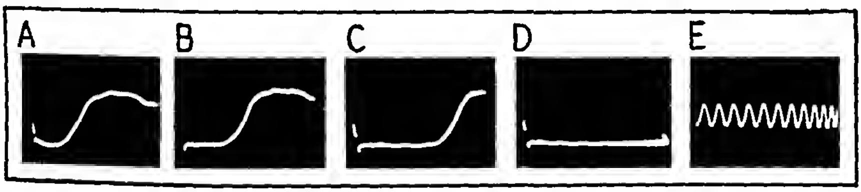

Fig. 9. Increase of delay produced by an anodal shock following a cathodal stimulus. Electrode arrangement as in figure 1C. The beginning of the records marks the time of application of the constant cathodal stimulus. The anodal shock followed after 3 msec.; its strength was gradually increased from A to D. E: 100 cycles.

cathodal shock was doubled the intensity of the anodal pulse had to be more than doubled for cancellation.

The description of interaction of two opposite shocks has dealt so far merely with cancellation of responses. When the intensity of the anodal shock was progressively increased, the interval being appropriate for cancellation, the first effect seen was a progressive lengthening of the stimulus-response delay (fig. 9). This gradual lengthening was quite similar to that which was obtained by progressively weakening a single cathodal shock (fig. 3).

All the phenomena reported in this section could be obtained not only when the two shocks were delivered through the same pair of electrodes, but also when three electrodes were employed, one of them common to the two stimuli (fig.

1B). Further aspects of the interaction of shocks of opposite polarity are described in the following section.

E. *Anodal stimulation.* By this expression is meant that invariably, when sufficiently strong shocks were applied, cardiac impulses could be initiated from the region of the heart in contact with the anode. The intensity of the shocks had to be from 1.2 to 3 times stronger than that necessary for cathodal stimulation.

These responses at the anode were not due to the "break" of the shocks—i.e., to the exponential subsidence of the condenser discharges. As is well known, the responses to the break of a current require higher intensities when the current is interrupted gradually than when it stops promptly. If in these observations the responses had been to the break of the shocks, then larger capacities, which subside gradually over a relatively long time, would have required greater voltage for anodal stimulation than that sufficient when smaller capacities were used, the discharge of which subsides promptly. The opposite, however, was observed. It is inferred, therefore, that the responses at the anode were stimulated by the "make" or onset of the pulses.

Responses at the anode to the make of a current have been often attributed to hypothetical neighboring physiological cathodes. The following observations were devised to test for the effectiveness of these assumed cathodes.

In some ventricles one of the stimulating electrodes was placed at a point closely equidistant from two recording leads. The diphasic record yielded by these leads was markedly influenced by minor shifts in the site of origin of an impulse arising between them, since the record depended on the relative time of arrival of the propagated disturbance at the two leads. Indeed, if the equidistant stimulating electrode was the cathode, shifting that electrode a fraction of a millimeter on the surface of the ventricle caused striking changes in the records. The records obtained with anodal shocks were as a rule quite similar to those which resulted from cathodal stimulation when the equidistant electrode was fixed (see the lower tracing in fig. 10). Furthermore, the responses to anodal stimuli were not changed when the anode remained fixed while the cathode was moved to different points. These results suggest that both the responses to cathodal and those to anodal stimuli arose at the point of the muscle in contact with the tip of the corresponding electrode.

In other observations the effects of a cathodal shock were studied on the response to a threshold anodal stimulus. Whether the two shocks were delivered through a single pair of electrodes, or with only one common electrode (fig. 1B), and whether the anodal stimulus preceded or followed the cathodal shock, the response could be cancelled. In other words, threshold cathodal and anodal shocks may cancel their stimulating effects even though the two shocks do not follow the same path in the ventricle. Figure 10 illustrates a typical instance.

Discussion. I. *The local response.* Katz (1937), Hodgkin (1938), and Pumphrey, Schmitt and Young (1940) have described an electric response of nerve, localized to the region of the cathode and occurring with subthreshold (as low as 50 per cent) as well as with threshold shocks. The experiments of Hodgkin

and those of Pumphrey, Schmitt and Young were similar to the observations illustrated in figure 5, that is, the records were taken from one of the stimulating electrodes and the effects of cathodal and anodal shocks were compared.

The asymmetry between the cathodal and the anodal records (p. 56, fig. 5) leads to the inference that in the heart, as in the nerves studied by these authors, there is an active, non-propagated, graded electric phenomenon elicited by electric stimuli. This phenomenon will be referred to as the local response.

Some differences between the local response of nerve and that of the ventricle may be mentioned. In nerve the local response is not seen with shocks less than 50 per cent of threshold for the propagated impulse. In the ventricle the

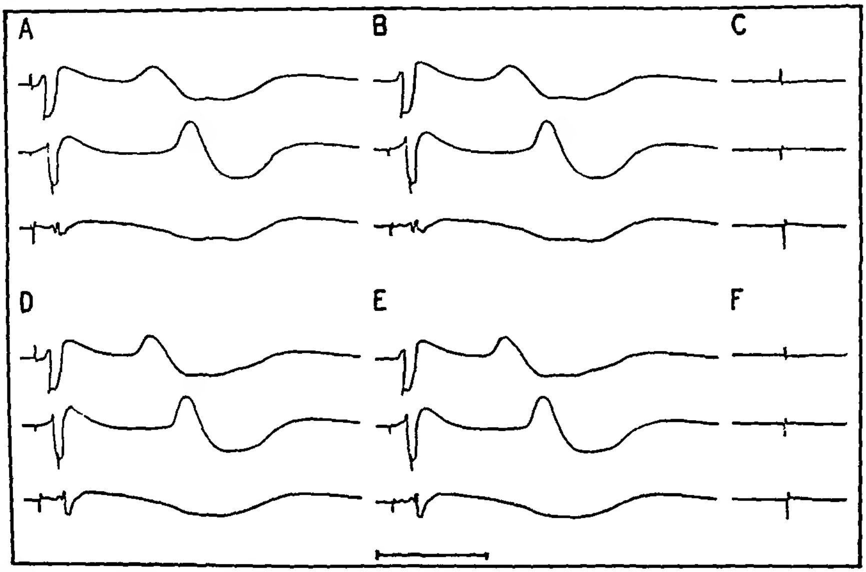

Fig. 10. Cancellation of the response to a threshold anodal shock by a threshold cathodal shock, and *vice versa*. The stimulating electrodes were placed as in the diagram in figure 1B, but the electrodes *b* and *c* were on crushed tissue. One shock (anodal) was delivered with the anode at *a* and the cathode at *b*; the other (cathodal) had the cathode at *a* and the anode at *c*. There were 4 recording leads, 3 of them referred to the 4th one.

A, anodal shock alone; B, cathodal alone; C, anodal followed (2.6 msec.) by the cathodal. D, E and F, after readjustment of the recording leads. D, anodal alone; E, cathodal alone, F, cathodal followed (2.6 msec.) by the anodal.

asymmetry between cathodal and anodal polarization records increased steadily when the shocks were strengthened. In nerve the local response can appear as a separate hump in the cathodal records; in the ventricle the cathodal tracings were approximately exponential, with no recognizable trough separating passive from active polarization (figs. 3 and 5).

II. *The stimulus-response delay.* It often has been denied that there may be a significant lapse of time between the application of a brief stimulus and the initiation of the propagated ventricular response. Thus, Blair, Wedd and Young (1941) state that the delay, if it exists at all, is a negligible part of the total latent period of a response recorded after conduction. On the basis of the present

data (figs. 2, 3 and 4) it is likely that Blair, Wedd and Young used unnecessarily strong suprathreshold shocks.

The observations described and analyzed on p. 53 (figs. 3, 4 and 5) leave no doubt that at or near threshold strength of the stimuli the delay can readily last more than 50 msec. Indeed, in preparations which had been exposed for several hours and had accordingly deteriorated, delays as long as 1 sec. were repeatably measured. The conduction velocity of these hearts was only slightly slower than when freshly excised.

The stimulus-response interval in nerve, according to Blair and Erlanger (1936), could be due to a delay of the rise of the stimulating potential because of the complicated structure of nerves as networks. Specifically, Blair and Erlanger suggest that if the shock charges a large leaky capacity which is in parallel with a resistance and the smaller capacity of the polarizable responding interface, then the maximum potential at the latter capacity would be late and could coincide with the time of response. This concept, which may be applicable to brief latencies, would require entirely improbable electrical values to explain the prolonged delays seen in the present observations.

Long ventricular latencies may be due in some instances to the initiation of impulses at some point distant from the stimulating cathode (cf. Hodgkin, 1938). The reasons have been given for dismissing this factor in the majority of the present observations (p. 53). It may be added here that, if this were the cause of increased delays, since an increase was invariably seen when the shocks were weakened from suprathreshold to just-threshold intensity, then the quite unlikely corollary would ensue that the weaker a shock, the further from the cathode the stimulated region.

III. *The intermediary process.* The fact that the stimulus-response delay can be long, leads to the inference that stimuli do not trip the spike-mechanism directly, but indirectly through an intermediary process. In the observations on nerve of Hodgkin (1938) and of Pumphrey, Schmitt and Young (1940) the spike always started at the peak of a local response. Similarly, in strips of cardiac muscle Bozler (1942) found that each spontaneous impulse was preceded by a period of slowly rising negativity, localized to the pacemaker. Such records suggest that the process responsible for the local response might be the process intermediary between the stimulus and the propagated impulse. Indeed, Pumphrey, Schmitt and Young not only proposed that the build-up of the local response to a critical level can be the tripping mechanism for the spike, but also that the important tripping factor is the local electrical potential, whether that potential be developed to the critical level by the active local response mechanism or by passive polarization due to an initial or to additional stimuli.

The present data do not support the applicability of the hypothesis of Pumphrey, Schmitt and Young to the ventricle. The responses need not begin at the peak of a rising potential; they may start at different times during the wane of the initial polarization, indeed, when that polarization has entirely subsided (figs. 3, 5 and 9). Furthermore, if the hypothesis were applicable to

the ventricle, then no matter when an anodal shock might be applied during the delay, it should be capable of canceling the response to a threshold cathodal stimulus by changing the polarization in the anodal direction. The existence of an interval in the delay period during which a response cannot be canceled (p. 58, fig. 8) opposes, therefore, the hypothesis.

The following suggestions emerge from the observations on the ventricle. The delay is due to the development of a process intermediary between the stimulus and the spike. Although the shocks elicit a local response (defined as above), this local electric phenomenon is not the intermediary process; the data do not throw any light on the possible rôle of the local response. The intermediary process does not have a significant electrical potential sign. Its rate of development to a critical tripping level is proportional to the strength of the stimulus—i.e., with strong shocks the process develops rapidly, hence a brief delay; with weak shocks it develops slowly, hence a long delay. The long delays which may ensue upon repeated stimulation (fig. 5) may be due to an influence of the relatively refractory condition of the ventricle on the rate of development of the intermediary process, or else they may indicate that the process has to build up to a higher tripping level when there is refractoriness. A subthreshold stimulus is followed by a subliminal intermediary process which is relatively long enduring, hence the possibility of summing the effects of repeated stimuli at long time intervals (p. 56). Even quite weak stimuli result in the buildup of a subliminal intermediary event, since test shocks applied later denote increased excitability (fig. 6).

Anodal stimulation is discussed below. If this action is neglected for purposes of simplification, the possibility of canceling the response to a cathodal stimulus by a following or preceding anodal shock (fig. 7) leads to the conclusion that an anodal shock opposes the development of the cathodal intermediary process. Since an anodal shock applied after a critical interval cannot cancel the response to a cathodal stimulus, it may be further inferred that the anodal influence prevents the development of the intermediary process only during the early part of that development. An alternative view which recognizes anodal stimulation would be as follows. The anodal shocks exert both a stimulating effect and an action which opposes the cathodal results. With relatively long intervals strong anodal shocks would be necessary for cancellation of the cathodal response because the intermediary process caused by the cathodal stimulus would be largely developed. Strong anodal shocks, however, would in turn elicit excitatory processes of their own which would lead to a response. Cancellation would not be obtained at long intervals, therefore, because the stimulating action of a strong anodal shock would predominate over its ability to abolish cathodal stimulation.

IV. *Anodal stimulation.* It is still an open question whether responses which arise upon the make of a current or a pulse in the neighborhood of the anode are due to anodal stimulation or are due to excitation at neighboring physiological cathodes—i.e., regions where current leaves the elements. Commenting on Rosenblueth's (1941) inference that there is anodal make stimulation in motor

nerves, Gerard (1942) states that the complicating possible action of spurious poles can hardly be dismissed on the evidence to date. We are in agreement with Gerard's statement. We would add, however, that the evidence to date does not suggest the existence of effective spurious poles, and that the burden of the proof should fall upon those who affirm their existence.

The data in section E support a direct stimulating action by the anode. If anodal stimulation were at a spurious cathode, it is difficult to understand why a second threshold cathodal shock cancels the action of the first anodal pulse when the two shocks have only one common electrode—i.e., when the currents corresponding to each of the shocks travel over largely independent intraventricular pathways (fig. 10). The second cathodal shock would be expected to sum its action with that at the initial spurious cathode, instead of opposing it. Two cathodal shocks applied with a similar arrangement of electrodes sum their effects (p. 57). The results become understandable, however, if it is assumed that the two stimulating effects occur immediately at the regions in contact with the physical common pole, and that a cathodal pulse can oppose the development of excitation by an anodal stimulus, much as an anodal pulse can oppose cathodal stimulation (p. 63).

SUMMARY

Over 50 msec. may elapse between the time of application of a brief electric shock and the beginning of the propagated response (figs. 2 and 3). The stimulus-response delay is influenced by the characteristics of the stimuli (fig. 4); it may vary in the course of a series of responses to constant stimuli (fig. 5).

The electric phenomena which precede a response at the site of origin are described (figs. 3 and 5).

Two successive shocks interact in their effects when they have the same (p. 56, fig. 6) or the opposite polarity (figs. 7 to 10). There is a period after the application of a threshold stimulus during which a following shock of opposite polarity may cancel the response (fig. 7); there is also a later period during which the response cannot be cancelled (fig. 8).

The data suggest that the activation of the propagated impulse by an electrical stimulus is not direct, but is exerted through an intermediary process (p. 62). This process is not analogous to that which yields "local responses" in nerve (p. 62).

"Make" stimulation at the anode is described (p. 60, fig. 10) and discussed (p. 63).

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CARDIOVASCULAR EFFECTS OF EXPERIMENTAL INSOMNIA

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Kleitman (1923) studied the physiological effect of 115 hours of experimental insomnia on the human subject, and concluded that "Respiration, heart rate, blood pressure showed a marked decrease in insomnia . . . ", attributing the decrease to the greater muscular relaxation of the sleepy subject. In two experiments on himself, the heart rates in the recumbent posture were reduced 16 and 17 per cent respectively, the systolic blood pressures 5 and 6 per cent, and the diastolics 6 and 8 per cent. Sitting, the rate was reduced only 7 per cent, while the systolic pressure increased 5 per cent and the diastolic increased 6 per cent. Of the several insomnia studies cited in a later review (Kleitman, 1929) only that of Herz (1923) included the cardio-vascular variables; in this experiment (involving a single subject) there was no reduction in the heart rate. Edwards (1941) reported that there were no significant changes in blood pressure or pulse rate as a result of the loss of 100 hours of sleep; no data were given and the criterion of significance was not mentioned.

Scott (1921) published several case reports showing substantial decrements in the Schneider (1920) cardio-vascular scores of military personnel who had had subnormal amounts of sleep. Possibly other factors were operative in producing the decrement; if not, the results are in disagreement with those obtained by Kleitman (1923) since reduction in pulse rate implies an increase in the Schneider index.

It was intimated by Kleitman that the lowered pulse rate found by him could have resulted from the tendency of the sleep-deprived subject to doze during rest while the counts were being made. In the experiment to be described, this hypothesis was tested by observation of the heart rate during one-minute standard exercises as well as at rest. The rest periods and exercises were ordered in a way that also made it possible to calculate several cardio-vascular test scores of current interest, including Schneider index scores.

METHOD AND PROCEDURE. The procedure was identical with that described by the writer in connection with another experiment (Henry, 1942). The subjects were rested in the reclining position, blood pressures were taken by auscultation, using a mercury manometer, and heart beats were recorded on a polygraph by amplified cardiac action potentials (Henry, 1937). Similar measurements were taken in the standing position, and heart beats were continously recorded during the exercise and for two and one-fourth minutes afterwards. Three exercises were given each subject using 18, 32 and approximately 33 thirteen inch stool mountings per minute. Twenty seconds after the third exercise, the subject maintained a positive intra-thoracic pressure of 20 mm. Hg as long as possible, while blowing through a Flarimeter (Wells, 1930). The tests were repeated twenty-four hours later under comparable conditions, the subject

refraining completely from sleep during the test-retest period. Eight male university students, aged 20 and 21, were tested. The experiment was conducted during the late afternoon, and no food was ingested for three hours preceding each test. The significance of the differences between experimental and control conditions was tested by conventional small-sample statistics, using Fisher's t (Peters and Van Voorhis 1940). Changes in the following functional tests were also examined:

a. The Crampton (1913) blood ptosis test, scored from systolic pressure and heart rate changes due to changing posture from reclining to standing.

b. The Foster (1914) physical efficiency test, scored from standing heart rate, the rise as a result of standard exercise, and the extent of recovery in 45 seconds.

c. The McCurdy (1910) condition test, scored from the heart rate increase when the posture is changed from reclining to standing.

d. The McCurdy-Larson (1935) organic efficiency test scored from resting diastolic pulse pressures, extent of negative or positive increment in heart rate two minutes after exercise compared with the resting rate, time that 20 mm. Hg intrathoracic pressure can be maintained 20 seconds after standard exercise and vital capacity.

e. The pulse-ratio test (Campbell, 1925) scored from the ratio of the two minute post-exercise heart rate to the one minute resting rate.

f. The Schneider (1920) cardiovascular rating, scored from the items of the Crampton test with the addition of heart rate immediately after standard exercise and rapidity of return to normal.

g. The Tuttle (1931) physical efficiency test, scored as the amount of exercise calculated to produce a 2.5 pulse ratio by linear interpolation from two pulse-ratio tests using different amounts of exercise.

Some of these tests were modified somewhat as to the amount of exercise, as otherwise all could not have been included in a one hour test battery.

Results at rest. No effects were found in systolic, diastolic, or pulse pressures, either reclining or standing, or in the change in pressure due to standing. The largest t was 1.50. The reclining pulse rate was 7 per cent lower under experimental conditions, a significant change since t was 2.7. The standing pulse rate was 4 per cent lower, but this was not significant since t was only 1.3.

Results during exercise and recovery. The average pulse rate during exercise was 5 per cent lower ($t = 3.0$) under experimental conditions for the first exercise, 5 per cent lower for the second ($t = 2.8$) and 6 per cent lower ($t = 3.1$) for the third. These differences are statistically reliable. During the 1st minute of recovery the rates were 7 per cent ($t = 1.4$), 9 per cent ($t = 2.4$) and 7 per cent ($t = 2.1$) lower; during the second minute of recovery they were 10 per cent ($t = 1.8$), 11 per cent ($t = 2.7$) and 5 per cent ($t = 1.0$) lower. The trend of the total recovery pulse was therefore consistent, and it was lowered by a statistically significant extent for the second exercise, but not for the first, which involved a relatively small amount of work, or for the third, where the results were complicated by the positive intra-thoracic pressure applied at 20 seconds post-exercise.

These heart-rate counts may be fractionated into fifteen second intervals (fig. 1) with very interesting results. The amount of decrease under experimental conditions becomes increasingly marked toward the end of the exercise periods and also toward the later stages of the recovery periods. There is also an increased negative phase in late recovery under experimental conditions. The differences tend to be more significant statistically during recovery from the two harder exercises (table 1).

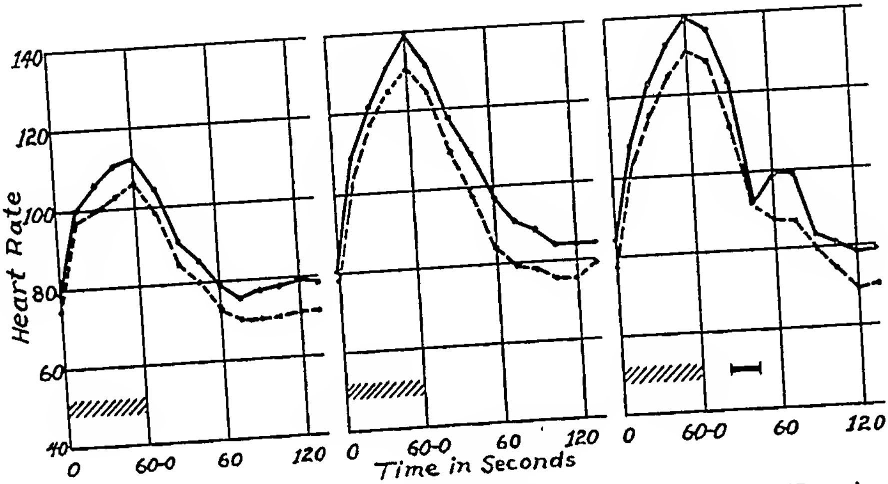

Fig. 1. Mean heart rate for 15 second periods plotted against time. Exercise periods are indicated by cross-hatching and the Flarimeter blow after the third exercise is marked by a solid bar. The broken-line curves were obtained during insomnia; the solid-line curves show the response of the same subjects under control conditions.

TABLE 1

Fisher's t for 15 second heart-rate counts

NO.	DURING EXERCISE				FIRST MINUTE RECOVERY				SECOND MINUTE RECOVERY				
	0–15″	30″	45″	60″	0–15″	30″	45″	60″	75″	90″	100″	120″	135″
1	1.1	2.6	3.9	2.2	1.4	1.3	1.4	1.3	1.2	1.8	2.0	1.9	1.8
2	2.1	2.4	2.5	3.0	1.8	1.9	2.9	2.9	3.2	2.3	2.3	2.4	1.4
3	2.2	3.2	2.8	3.2	2.4	1.6	0.1	2.3	3.8	0.7	1.8	3.4	2.3

Note: Recovery in the third exercise is complicated by the Valsalva experiment. A t of 2.3 is equivalent to a probability of 95/100 in favor of a real difference, which Fisher considers statistically significant (Peters and Van Voorhis, 1940, pp. 173 and 176).

The effects of the modified Valsalva experiment (i.e., the post-exercise Flarimeter blow) can be studied by examining the differences in heart rates between corresponding parts of the recovery for the second and the third exercises. These differences are shown in table 2. The Flarimeter blow began 20 seconds after the end of the third exercise, had an average duration of 23.3 seconds for the controls, and was shortened only 0.8 second under the insomnia conditions. Inspection of individual records reveals that typically there is a relative brady-

cardia[1] as the subject nears the end of his blow. Immediately after the blow there is a period of relative tachycardia lasting about 20 seconds, followed by a second period of relative bradycardia that may last a minute or more.[2] Under conditions of insomnia, the relative slowing of the heart during the blow is only about a third as great, and the onset of the second or post-blow phase of slowing is considerably delayed. There is no significant alteration in the degree of relative tachycardia or second phase bradycardia.

Results with cardiovascular functional test scores. These are given in table 3. It will be seen that only the Crampton test shows a decrement as a result of the insomnia and this is probably not statistically significant. The Foster and

TABLE 2

Valsalva effect

Mean differences between second exercise and third exercise recovery heart rates

	SECONDS AFTER END OF EXERCISE								
	0–15″	30″	45″	60″	75″	90″	105″	120″	135″
Control	5.4	4.9	−16.4	2.9	8.6	−4.9	2.9	−5.7	−5.4
Insomnia	4.3	2.7	−5.7	2.6	7.7	1.9	−1.0	−5.8	−8.9
t	0.9	1.2	3.4	0.5	0.4	3.0	1.9	0.2	2.0

Note: Heart rates are in beats per minute. The flarimeter blow occurred in the 30″ and 45″ periods. A negative sign indicates a relative bradycardia.

TABLE 3

Mean cardiovascular test scores

	CRAMPTON	FOSTER	MCCURDY	MCCURDY-LARSON	PULSE-RATIO	SCHNEIDER	TUTTLE
Control	47.8	4.6	19.5	298.0	−2.67	9.3	31.8
Insomnia	40.0	7.0	22.8	324.0	−2.53	10.9	35.7
t	1.8	4.2	1.6	2.1	3.3	1.3	0.8

Note: A minus sign has been affixed to the mean pulse-ratio score so that a positive difference indicates improved condition of the subjects as in the other tests.

pulse-ratio tests, being heavily loaded with the recovery heart rate, show significant increments. Improvement in the McCurdy-Larson test approaches significance.

Conceivably the experimental conditions could have altered motivation, thus changing vital capacity and the toleration time for positive intra-thoracic pressure, but the changes were not significant (t = 0.9 and 0.5). A modified U-tube

[1] Relative bradycardia, as the term is used here, is intended to mean that the heart rate is slower in a given interval of the third exercise recovery period than in the corresponding interval of the second exercise recovery period.

[2] Records obtained in other experiments (Henry, 1942) have also been examined, and quite uniformly show that this is the usual course of the post-exercise post-blow heart rate, when the blow is maximal in length as in the present case.

Manometer Test (Warner and Hambly, 1925) using 20 mm. pressure instead of the usual 40 mm., also failed to give significant changes ($t = 1.0$).

DISCUSSION. The positive results with reclining heart rate and negative results with blood pressures and the standing rate are explainable on the basis of Kleitman's (1923) hypothesis of "greater muscular relaxation of the sleepy subject" and a tendency on the part of the subject actually to sleep while reclining. The results during exercise and recovery cannot be explained so simply. The effects of insomnia to be accounted for are:

a, unaltered standing heart rate at rest;
b, probably unaltered heart rate during the first 15 seconds of exercise;
c, significantly depressed heart rate during the last 45 seconds of exercise, and during recovery from the more severe exercises;
d, probably increased negative phase in recovery;
e, lessened relative bradycardia during the Flarimeter blow;
f, unaltered relative tachycardia following the blow;
g, probably unaltered but delayed relative bradycardia following the blow.

Considering the temporal position of the alterations in rate due to insomnia, it seems possible that a lessened irritability has occurred in some part of the mechanisms responsible for the normal increase in heart rate toward the end of exercise and during recovery. It is also possible that a lessened irritability is involved in the smaller amount of relative bradycardia during the Flarimeter blow, since there is evidence that chemical factors are important in the terminal phase of the blow when it is made during recovery from exercise (Henry, 1942). It is doubtful if increased vagotone is much of a factor, as there seems to be no significant reduction in heart rate during the parts of the experiment in which the rate is known to be primarily controlled by the vagus (Dawson, 1935, pp. 254 and 487), namely, *a*, *b* and *f* above. The postulation of reduced accelerator tone (Dawson, loc. cit., also p. 243) seems to account for most of the experimental findings, although it offers no explanation for *e* or *g*. Naturally, further data are required before any theoretical explanation can be more than speculative.

It appears obvious that loss of sleep must be considered in the interpretation of functional test scores that involve pulse rate. Insomnia seems to present a special case when reduction in the pulse rate is associated with impaired, rather than improved, physical condition. This anomalous effect is greatest during exercise and the later stages of recovery, and is probably quite small under resting conditions.

Flinn (1941) observed a decrement of about 5 per cent in the pulse rate of truck drivers as a result of 0.1 to 9.9 hours of driving since the last major sleep, but was unable to offer an adequate explanation for the reduction. In the light of the present study, it seems quite possible that sleep loss may have been a causal factor in his experiment.

SUMMARY AND CONCLUSIONS

The mean heart rate of eight male human subjects was lowered at rest in the reclining posture but not while standing, as the result of 24 hours of sleep de-

privation. It was also lowered during exercise and recovery. An increased negative phase was observed, and there was also a reduction in the amount of relative bradycardia produced by a modified Valsalva experiment performed during early recovery from exercise. A reduced irritability of some part of the mechanisms responsible for rate control during late exercise and recovery is postulated in explanation.

Scores on the Foster, pulse-ratio, and McCurdy-Larson cardiovascular tests were markedly improved as a result of the insomnia. The Schneider index was also raised, but the increase was not statistically significant.

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DECREASED FAT APPETITE PRODUCED IN RATS BY LIGATION OF THE COMMON BILE DUCT

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In previous self-selection studies it was found that removal of most of the pancreatic tissue from rats, leaving the bile duct intact, resulted in a marked increase in the appetite for fat and a decrease in the appetite for carbohydrate. By virtue of the satisfaction of these appetites the usual diabetic symptoms, polydipsia, polyphagia, hyperglycemia, and loss of weight, did not develop (Richter and Schmidt, 1941).

It became of interest to determine whether ligation of the bile duct leaving the pancreatic tissue intact would produce any changes in appetite, especially for fat and carbohydrate.

METHODS. The experiments were performed in two series. In the first the rats had access to butter and to a mixed diet which contained a high amount of carbohydrate and no added fat. In the second series the rats had access to a full self-selection diet—11 different substances including a fat and a carbohydrate, all offered in separate containers.

In the first series the rats were kept in separate cages which contained two food cups and one graduated inverted water bottle. One food cup contained saltless butter, the other our stock diet mixture without butter. In this mixture carbohydrate constituted 58.3 per cent, and fat 1.9 per cent. Thus the rats had a choice between a fat and a diet which contained a very high amount of carbohydrate. Each rat had access also to a graduated inverted bottle filled with tap water.

Records of the intake of the butter, stock diet and water were made daily; body weight was recorded weekly. In order to obtain base lines, records were taken for several weeks before the bile duct was ligated.

In the second series the rats were placed separately in cages equipped with 3 food cups and 8 graduated inverted bottles, containing the following substances:

In food cups. 1. Casein (purified). 2. Dextrose, C.P. 3. Yeast (dried brewer's) or powdered whole liver.

In graduated inverted bottles. 4. Olive oil. 5. Cod liver oil. 6. Sodium chloride, 3 per cent. 7. Dibasic sodium phosphate, 4 per cent. 8. Potassium chloride, 1 per cent. 9. Magnesium chloride, 0.5 per cent. 10. Calcium lactate, 2.0 per cent. 11. Tap water.

From these substances normal rats make selections in such a manner that excellent growth and development are maintained.

Here again the intake of each substance was recorded daily and body weight weekly. The mineral solutions, olive oil and cod liver oil were changed twice

weekly. Records were taken for at least 20 days before the bile ducts were ligated.

At operation the bile duct was exposed through a midline abdominal incision about two centimeters long starting just below the xiphoid process. The ligatures were placed either near the duodenum, near the liver or midway between the liver and the duodenum. Since it was found (Cameron and Oakley, 1932; Richter and Benjamin, 1934) that single ligatures usually produce only a temporary obstruction, the bile duct being able to absorb the ligature and recanalize, in most instances we placed two ligatures on the duct and made a cut between them. From the results of previous experiments we knew also that after ligation the bile duct becomes distended, only mildly in some rats, very markedly in others.

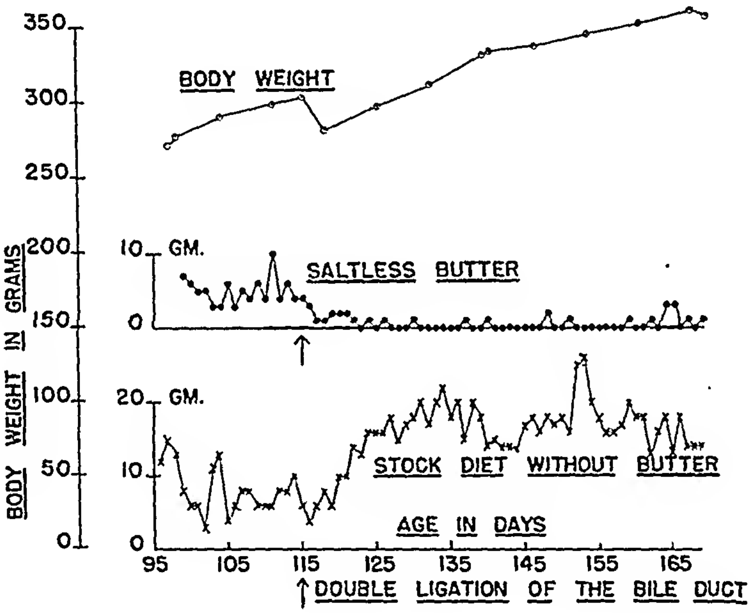

Fig. 1

In the present experiments bile ducts were either singly or doubly ligated in 25 rats. Of this number 6 died within the first 10 days with ruptured bile ducts, and their records are not included in the present paper. The mortality was greatest in the group of rats with double ligation and section near the duodenum. It was lowest in the rats with double or single ligation above the upper pancreatic duct, that is, fairly close to the liver.

At autopsy we recorded chiefly the presence or absence of distention of the duct and of bile fluid in the abdominal cavity.

Results. Figure 1 gives the record of one of the 6 rats used in the first series. This rat was placed in the experimental cage at an age of 95 days. For 10 days before operation its daily intake of saltless butter averaged 5.0 grams, and of the butterless stock diet 7.2 grams. When the animal was 115 days of age the bile duct was doubly ligated and cut near the liver. On the following day the rat ate less butter and after 7 days scarcely touched it. In marked

contrast it began to eat much more of the stock diet and after 10 days ate more than twice as much per day (16 grams) as before. At the end of 59 days, when it was killed the bile duct showed a mild degree of distention with a capacity of approximately 2 cc., but there was no jaundice.

Table 1 summarizes the results of the observations made on the 6 animals in this series. In 4 of these rats the bile ducts had been doubly ligated and cut near the liver, and in two singly ligated near the liver. Three rats with a double ligature died in 15, 24, and 25 days, respectively, and one with a single ligature died in 15 days. One rat with a double ligature near the liver was killed at the end of 59 days; another rat with a single ligature was killed after 52 days. At autopsy all of the rats showed moderate to marked distention of the bile ducts.

The table gives the average daily intake records for the last 10 days before the operation and for the last 10 days before the rat died or was killed. Usually for the first 3 to 8 days postoperatively the rats ate only small amounts of either the stock diet or of the fat. Then their appetites returned and their food-

TABLE 1

Average daily intake for last 10 days before bile duct ligation and last 10 day period before death

RAT NO.	OPERATION	SURVIVAL TIME	SALTLESS BUTTER		BUTTERLESS FOOD		WATER	
			Before	After	Before	After	Before	After
		days	*grams*	*grams*	*grams*	*grams*	*cc.*	*cc.*
1	Double near liver	59 K	5.0	0.2	7.2	18.1	20.7	28.0
2	Double near liver	25	3.9	0.7	10.3	13.6	26.4	32.6
3	Double near liver	24	4.7	0.3	5.5	11.1	14.6	25.6
4	Double near liver	15	4.7	1.0	7.2	7.5	20.8	18.9
5	Single near liver	15	4.3	0.6	8.2	9.1	18.6	23.7
6	Single near liver	52 K	2.5	1.4	10.2	14.8	23.3	26.9
Average................		31.7	4.2	0.7	8.1	12.4	20.7	26.0

intake quickly reached a plateau. For the 6 rats the average daily intake of butter decreased from 4.2 grams for the last 10 days before operation to 0.7 gram for the last 10 days of life. In the same periods the intake of the stock diet with the high carbohydrate content increased from 8.1 to 12.4 grams; and water intake increased from 20.7 to 26.0 cc.

In the second series of experiments the rats had access to 11 different substances. From previous experience we know that when the vitamin B complex is offered to rats in the form of yeast or liver powder, some of the rats will take large amounts of fat and little or no carbohydrate, while others take large amounts of carbohydrate and little or no fat. In previous publications we have referred to the former as "carbohydrate burners", the latter as "fat burners". The reasons for these differences are not clearly understood but also are not relevant to the present purposes (Richter, Holt and Barelare, 1938). In the present series 7 rats were "fat burners" and 5 "carbohydrate burners" before operation. The observations on the two groups will be reported separately.

Figure 2A gives a record for one of the "fat burners". The record shows the intake of casein, dextrose, liver, and olive oil for 20 days preoperatively and until the rat died 36 days postoperatively. For the 20 days before operation the rat took large amounts of olive oil, smaller amounts of liver, and little or no casein or dextrose. On the day following double ligation and section of the bile duct near the liver the rat took no olive oil and from then on until it died it took only minimal amounts, during the last 20 days scarcely touching either olive oil or cod liver oil. In contrast it indicated a marked appetite for dextrose, which, however, did not manifest itself until quite suddenly 9 days postoperatively, when there was a sharp increase to an average of 5.9 grams per day, at which it remained until a day or two before the death of the animal. The casein appetite remained very low and the liver powder appetite decreased irregularly. The rat did not show definite changes in appetite for any of the other substances. At autopsy the bile duct was very much distended, filling approximately one quarter of the abdominal cavity.

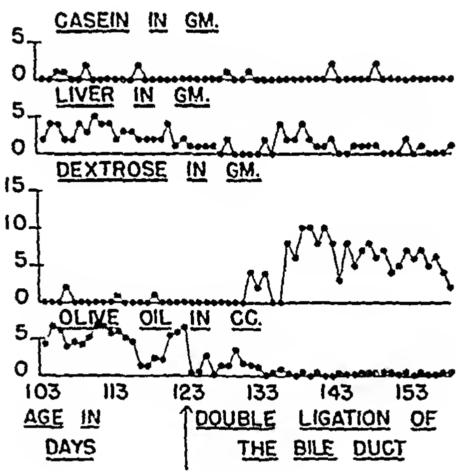

Fig. 2A

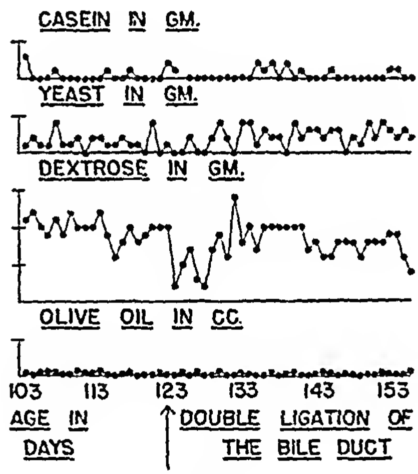

Fig. 2B

Table 2 summarizes the results of the observations made on the 7 "fat burners" and the 5 "carbohydrate burners". In 4 of the former group the bile duct was doubly ligated and cut approximately half-way between the liver and the duodenum; in one near the duodenum; in two near the liver. The survival times varied from 11 to 37 days. All 7 rats had well distended bile ducts. The table gives the average daily intake of the different substances for the last 10 days before operation and also for the last 10 day period before the rat died or was killed. The average fat intake (olive oil plus cod liver oil) decreased from 3.5 to 0.9 cc. per day after operation, with all 7 rats showing a definite decrease; the average dextrose intake increased from 2.0 to 2.9 grams, all except one rat either maintaining the preoperative level or showing an increase; the casein intake remained essentially the same while the yeast or liver intake decreased markedly in all 6 animals.

Figure 2B gives a record of one of the "carbohydrate burners". The bile

duct was ligated when the rat was 123 days of age. Postoperatively it took less dextrose for a few days, but after that continued to take high amounts up to the last day before death. It continued to take only minimal amounts of olive oil and showed a slightly increased appetite for yeast, while the casein intake remained unchanged. It was killed after 34 days. The bile duct was moderately distended, and the subcutaneous tissues showed some jaundice.

TABLE 2

Average daily food intake for 10 days before bile duct ligation and for last 10 days before the rats died or were killed

RAT NO.	OPERATION	SURVIVAL TIME	OLIVE OIL PLUS COD LIVER OIL		DEXTROSE		CASEIN		LIVER		YEAST	
			Before	After	Before	After	Before	After	Before	After	Before	After
	A. "Fat burners"											
		days	*cc.*	*cc.*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
7	Double midway	11	3.1	0.9	0.7	1.6	0.2	1.8	3.6	0.8		
8	Double midway	15	2.4	0.4	2.4	2.8	2.8	1.5	1.6	0.7		
9	Double midway	14	5.7	1.4	0.2	0.4	0.5	0.6			1.4	0.1
10	Double near duodenum	27	2.6	1.0	4.0	6.2	2.0	1.3			1.0	0.6
11	Double near liver	37	4.5	0.7	0.2	6.1	0.2	0.4	2.5	0.6		
12	Double midway	24	2.3	1.4	6.7	3.1	0.4	0.7	2.1	0.7		
13	Double near liver	11	3.9	0.6	0.1	0.2	0.4	0.7	2.5	0.1		
Average		19.9	3.5	0.9	2.0	2.9	0.9	1.0	2.5	0.6	1.2	0.4
	B. "Carbohydrate burners"											
14	Double near duodenum	17	0.7	0.5	8.0	3.3	2.9	0.6			1.8	0.4
15	Double near duodenum	34	0.7	0.8	10.8	9.7	0.2	0.0			2.3	2.2
16	Double midway	34 K	0.6	0.5	9.2	9.2	0.4	0.8			1.3	2.4
17	Double near duodenum	19	0.7	0.6	9.1	7.1	1.1	0.4			1.0	1.2
18	Double near liver	24	0.6	0.7	7.9	8.6	3.0	1.6	1.2	0.8		
Average		25.6	0.7	0.6	9.0	7.6	1.5	0.7	1.2	0.8	1.6	1.6

Table 2B summarizes the results of the observations made on the 5 "carbohydrate burners". The bile ducts of three rats were doubly ligated or cut near the duodenum, in one other about midway between the duodenum and the liver, and in a fifth near the liver. The survival times varied from 17 to 34 days, at which time the last remaining rat was killed. In this group of rats which started with a very low fat intake, the fat appetite remained on the same low level after operation. With one exception all of these rats continued to eat considerable amounts of dextrose until within a few days of death. The average daily intake decreased only from 9.0 grams before operation to 7.6 grams

after operation. The fat intake of all 5 rats averaged 0.7 and 0.6 cc. respectively before and after ligation of the bile duct. The average casein intake decreased from 1.5 to 0.7 gram, with four rats showing a decrease; in three of the five rats the yeast and liver intake decreased.

The rats did not show consistent changes in appetite for any of the other substances with the possible exception of a slightly decreased appetite for sodium phosphate and magnesium chloride.

The results of these self-selection experiments are in agreement with the first series in showing that after bile duct ligation the rats did not take fat, except possibly in very small amounts, but did take carbohydrate. With only two exceptions they showed a reduced appetite also for yeast or liver powder.

Discussion. It is a well known clinical observation that the ingestion of fatty foods precipitates attacks of biliary colic in patients with bile duct obstruction. The results of the present self-selection experiments indicate that rats avoid fat even when their bile duct is occluded in such a manner that the external pancreatic secretions still are permitted to flow into the duodenum. When bile is absent the rats make selections which tend to eliminate fat from the diet, but maintain their caloric intake at an adequate level by consuming larger quantities of carbohydrates. After subtotal pancreatectomy the rats consumed large quantities of olive oil and absorbed it in the absence of a normal secretion of pancreatic enzymes. It seems likely that bile plays a predominant rôle in fat absorption, but whether it depends on a preliminary splitting of the fat by pancreatic enzymes or not is impossible to say from our experiments, since we have not been able to maintain totally pancreatectomized animals more than a very few days.

In these experiments we found another instance of the inverse relationship between the appetite for carbohydrate and fat. Thus far in all of our self-selection studies it has been found that the appetites for these two substances consistently show an inverse relationship. Thus, for example, in conditions of vitamin B deficiency rats refuse carbohydrate and eat fat. Placed on a high vitamin B diet they eat high amounts of carbohydrate and little or no fat (Richter, Holt and Barelare, 1937). In experimental diabetes produced by partial removal of the pancreas, rats refuse carbohydrate and select fat (Richter and Schmidt, 1941). Here we have seen that the rats with bile duct ligations eat little or no fat, but moderate amounts of carbohydrate.

In previous self-selection experiments we were able to show that the choices made by the rats were beneficial, that is, after various glandular or other deprivations they helped to maintain a constant internal environment, and so to prolong life or at least alleviate symptoms of disturbed metabolism. In the present experiment we have been able to demonstrate that after bile duct obstruction rats have an appetite for carbohydrate but not for fat. These choices are in agreement with clinical and experimental experience. However, we were unable to show that rats which could make their own selections did better, lived longer, etc. than those which are forced to take their food in certain fixed proportions. We did observe that some of the rats which completely refused

fat and ate only high amounts of carbohydrate had only very slightly dilated bile ducts as long as four weeks after double ligation and section; whereas, in a previous experiment in which the rats received only the stock diet with butter, we found that at the end of such periods all rats on the stock diet had markedly dilated bile ducts.

SUMMARY

1. Following ligation of the bile duct, 6 rats which were given access to butter and to a mixed diet with a low fat and a high carbohydrate content (1.9 per cent and 58.3 per cent respectively) stopped eating the butter and increased their intake of the mixed diet.

2. Following ligation of the bile duct, 11 rats which had access to casein, dextrose, olive oil, cod liver oil, 5 mineral solutions, and yeast or liver powder, ate little or no fat, but large amounts of the carbohydrate, dextrose. They ate less yeast or liver powder than before operation, and showed no definite changes in appetite for the casein or for the mineral solutions.

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CREATINURIA IN MAN FOLLOWING THE ORAL ADMINISTRATION OF CAFFEINE[1]

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An increase in the excretion of creatine has been observed in the urine of rats (1, 2) and of rabbits (3) following the parenteral injection of caffeine. In a study of the action of this drug on the metabolic processes in man we have frequently found creatine in the urine after oral administration. These observations were but one phase of a more comprehensive study of the effect of caffeine on metabolism as shown by the respiratory exchange and the urinary constituents. Data on the respiratory exchange have been reported elsewhere (4). The purpose of the present study is to show the amount of creatine and the frequency of its appearance in the urine with different doses of caffeine and to determine whether there was any relationship between creatinuria and the respiratory exchange, carbohydrate oxidation and protein metabolism.

Method. Three male adults served as subjects. Each customarily used coffee in moderation but abstained from all caffeine-containing beverages for twenty-four hours before the experiment. On the morning of the experiment the subject came to the laboratory in the fasting state and reclined for 30 to 45 minutes to recover from the small amount of exercise he had taken. The bladder was then emptied and the urine discarded. The subject then reclined for a basal period of 45 minutes, at the conclusion of which the urine was collected for analysis. This collection will hereafter be designated as the "post-absorptive" urine. Caffeine alkaloid dissolved in 200 cc. water at body temperature was then taken and, in the control experiments, an equal volume of water. The amount of caffeine ingested ranged from 0.6 to 6.0 mgm. per kilo body weight. The recumbent position was resumed for one hour and forty-five minutes and at the end of this time the urine was collected for analysis. The gaseous exchange was measured throughout the post-absorptive and post-ingestion periods in the manner described previously (5).

Creatine was determined by Benedict's method (6), the picric acid used in the analysis having been purified according to his directions (7). The data obtained are taken to be true values of creatine since Beard (1) using the enzyme of Miller and Dubos has shown that the chromogenic reaction in urine is specific for creatinine.

Results. The hourly urinary excretion of creatine for a period of one and three-quarter hours in all the experiments is shown in table 1. When the amount of caffeine ingested was 1.8 mgm. or more per kilo body weight, creatine appeared in the urine with but few exceptions. One milligram per kilo, however,

[1] Preliminary report: Federation Proceedings, March 16, 1942, p. 4.

induced creatinuria in only 3 out of 14 experiments. In two other experiments creatine was excreted in the post-ingestion period but these are not included in the table since it was present also in the post-absorptive urine. In none of the 8 experiments with 0.6 mgm. caffeine per kilo was there any creatine in the urine. Approximately one milligram caffeine per kilo therefore appears to be the minimal amount capable of inducing creatinuria in our subjects.

Inspection of the data in table 1 shows that there was no direct relationship between the amount of creatine excreted and the amount of caffeine ingested. The average hourly excretion was slightly less with 1.8 than with 1.0 mgm. caffeine per kilo and also less with 6.0 than with 3.0 or 3.6 mgm. The maximum excretion was approximately the same with 1.0 as with 1.8 mgm. caffeine and less with 6.0 mgm. than with 3.0 and 3.6 mgm. per kilo.

Creatine excretion and the respiratory exchange. Coefficients of correlation were calculated for the hourly excretion of creatine and 1, the increase in oxygen consumption above the basal level; 2, the increase in carbon dioxide elimination; 3, the total oxygen consumption; and 4, the total carbon dioxide elimination during one and three-quarter hours after the ingestion of caffeine. There was no significant correlation between the amount of creatine excreted and the respiratory exchange in terms of either the increase above the basal or of absolute amounts of oxygen consumed or carbon dioxide eliminated. The coefficients of correlation ranged from 0 to 0.10. Furthermore, there was no relationship between the occurrence of creatinuria and the height of metabolism. Since there was an increase in oxygen consumption and carbon dioxide elimination with the larger doses of caffeine, it is obviously necessary to limit comparisons of the gaseous exchange to those experiments in which the same dosage of caffeine was used. Such comparisons showed that in the different groups of experiments with various doses of caffeine, the range of increase in oxygen consumption and carbon dioxide elimination either as absolute values or as percentages above the basal was the same whether or not creatinuria occurred.

TABLE 1

Urinary excretion of creatine after ingestion of various amounts of caffeine

CAFFEINE INGESTED PER KILO BODY WEIGHT	NUMBER OF EXPERIMENTS	NUMBER OF EXPERIMENTS WITH CREATINE IN THE URINE IN P-E PERIOD	RANGE	HOURLY EXCRETION OF CREATINE (AVG. OF ALL EXPTS.)	HOURLY EXCRETION OF CREATINE (AVG. OF EXPTS. IN WHICH CREATINE WAS EXCRETED)
mgm.			*mgm.*	*mgm.*	*mgm.*
None*	10	0		0	0
0.6	8	0		0	0
1.0	14	3	0.9–1.4	0.4	1.2
1.8	6	4	0.6–1.5	0.7	1.0
3.0	12	11	0.8–4.7	1.6	1.7
3.6	7	7	1.5–4.2	2.2	2.2
6.0	10	8	0.7–2.2	1.0	1.4

* Control experiments.

As shown elsewhere (4) a rise in the respiratory quotient occurred after the ingestion of caffeine and reached the highest levels with the largest dosage. A critical study of the respiratory quotients of the individual experiments revealed, however, that there was no relationship between the presence of creatine in the urine and the height of the respiratory quotient. As in the case of the respiratory exchange it was necessary to limit comparisons to experiments with the same dosage of caffeine. A study of the individual non-protein respiratory quotients showed that there was no uniform or significant differences between the quotients in the experiments with and without creatinuria.

Carbohydrate metabolism. The presence of creatine in the urine was not related to the amount of carbohydrate oxidized. In those experiments in which creatine appeared in the urine after the ingestion of caffeine the total carbohydrate oxidation was not appreciably different than in those in which creatinuria did not occur, or in the control experiments in which water alone was taken. After the ingestion of 3 mgm. and 6 mgm. caffeine per kilo there was a marked rise in the respiratory quotient during the first and second periods after ingestion, but this rise was due to hyperventilation and not to an increase in carbohydrate metabolism (4). It was later followed by a fall of the quotient below the base line because of a compensatory retention of carbon dioxide. Notwithstanding the hyperventilation, calculations of the amount of carbohydrate oxidized on the basis of the respiratory exchange for the entire post-ingestion period of $1\frac{3}{4}$ hours were doubtless correct inasmuch as the compensation by retention of carbon dioxide was completed at the end of this period.

Creatinuria and nitrogen excretion. The procedure followed in these experiments was the same as in numerous experiments on metabolism conducted in our laboratories. Under these conditions we have found that the nitrogen excretion is almost invariably less in the post-experimental than in the post-absorptive period. Inspection of the average excretion in the different groups of the caffeine experiments indicated a trend toward smaller differences between the post-absorptive and post-experimental excretion of nitrogen as the dosage of caffeine was increased. A statistical analysis of the data showed that in those experiments in which creatine was found in the urine the decline in nitrogen excretion during the post-experimental period was significantly less than when there was no creatine present. In the experiments with no creatine in the urine, the average hourly nitrogen excretion was 108 mgm. less in the post-experimental than in the post-absorptive period whereas in the experiments with creatinuria it was 15 mgm. less. The difference between these two values (93 mgm.) was statistically significant as it was four times the sigma of the difference.

Discussion. Since there was no relationship in these experiments between the presence or the amount of creatine in the urine and the oxygen utilization or carbon dioxide elimination, it appears that the underlying mechanism responsible for creatine excretion following the ingestion of caffeine is not related to an increase in total metabolism. In view of these findings, may not the creatinuria observed in hyperthyroidism (cf. 8, p. 247) be merely incidental and not due to the heightened metabolism?

Brentano (3) found that the injection of 2 mgm. caffeine per kilo body weight

into rabbits was followed by creatinuria which paralleled a diminution in the glycogen content of the skeletal muscles. From these and other experiments he concluded that those conditions which lead to a reduction in muscle glycogen result in the excretion of creatine. Our experiments offer no direct evidence with regard to the glycogen content of the muscle before and after the administration of caffeine, but if a diminution in muscle glycogen did occur, it was not reflected either in a rise in blood lactic acid (5) or in an increased oxidation of carbohydrate.

Creatinuria induced by the ingestion of caffeine could conceivably result from an increase in the rate of blood flow with the consequent washing out of creatine from the tissues. Although the rate of blood flow in these experiments is not known, in view of the results obtained by Beard and his associates on rats (1, 9) it may be assumed that this factor was not responsible for the creatinuria. These observers found that after parenteral injection of caffeine the increase in the amount of creatine in the urine was accompanied by an increase and not by a decrease in the creatine content of the muscle. Creatinuria could perhaps be the consequence of diuresis but this did not occur in our experiments. In all the experiments the hourly excretion of urine was less in the post-experimental than in the post-absorptive period.

The larger amount of nitrogen in the post-experimental relative to the post-absorptive urine that was found when creatinuria occurred, could have resulted from an increase in the total volume of urine excreted or from stimulation of protein metabolism. Analysis of the data showed that there was no significant difference in the total volume of urine in the experiments with and without creatinuria. In the former the average hourly excretion was 14 cc. less in the post-experimental than in the post-absorptive period, while in the latter it was 56 cc. less. The difference between these two values was only 1.8 times the sigma of the difference. The difference between the nitrogen excretion in the experiments with and without creatinuria was statistically significant. Likewise, the difference between the excretion in the control experiments with water and with caffeine when the dosage exceeded 1 mgm. per kilo was significant. It appears therefore that the smaller difference between the post-absorptive and post-experimental excretion of nitrogen associated with creatinuria must be attributed to a stimulation of protein metabolism by caffeine.

The increase in protein metabolism may have been merely coincidental with the creatinuria or may have been a causative factor. While no definite conclusion can be drawn from the data at hand, it appears probable that the effects produced by caffeine on protein metabolism and creatine formation (or elimination) may represent two unrelated actions of the drug. This conclusion is suggested by a comparison of the results of these experiments with those obtained after the ingestion of 50 grams glucose or the same amount of fructose 10, 11). Creatinuria was induced almost invariably by the ingestion of fructose but rarely by the same amount of glucose. There was, however, no increase in protein metabolism (nitrogen excretion) in those experiments in which there was creatinuria, as compared with those in which no creatine was excreted.

As stated previously, there was no correlation in these experiments with

caffeine between the amount or presence of creatine in the urine and the total amount of carbon dioxide eliminated. However, after administration of the larger doses of caffeine which usually produced creatinuria, there was a blowing off of preformed carbon dioxide which was compensated for by retention before the conclusion of the experiment (4). In the experiments with fructose there was likewise a loss of non-metabolic carbon dioxide as a result of the formation of lactic acid (5). In both instances it may be reasonably assumed that there was a temporary alkalosis of the tissues. This common factor in the experiments with caffeine and with fructose would suggest that alkalosis may operate as a fundamental mechanism in the formation or liberation of creatine. It must be admitted, however, that different mechanisms might account for creatine formation under various experimental and pathological conditions.

CONCLUSIONS

The administration of sufficiently large doses of caffeine was followed by the excretion of creatine in the urine.

The minimal dose of the drug that induced creatinuria was 1 mgm. per kilo, but with this amount creatine was excreted in only 3 out of 14 experiments. With doses of 1.8 to 6.0 mgm. caffeine per kilo, creatine was found in the urine in 30 out of 35 experiments.

The amount of creatine excreted showed no relationship to the amount of caffeine ingested.

There was no correlation between the amount of creatine in the urine and total oxygen consumption and carbon dioxide elimination; nor was there any relationship between the occurrence of creatinuria and the gaseous exchange.

The presence of creatine in the urine was not related to an increase in carbohydrate oxidation.

An increase in protein metabolism was associated with creatinuria but it appears that it was not a causative factor.

While it is recognized that different mechanisms might account for creatine formation under various experimental and pathological conditions, these experiments in conjunction with others on fructose suggest that alkalosis might play a significant rôle in some instance.

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THE MOTOR INNERVATION OF THE COLON

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The response of the colon to stimulation of its various motor nerves has been the subject of many investigations. An adequate summary of the existing information is to be found in the review by Garry (1). Most of these studies have been concerned primarily with the type of activity (i.e., contraction or relaxation) of the colon which occurs in response to nerve stimulation. However, there are a number of equally important aspects of the motor innervation of the colon about which there is little or no information.

Certain of these issues are as follows: 1. The chemical mediators of the nerve impulse from the various autonomic nerves to the musculature of the colon have not adequately been determined. Such information is necessary for a proper functional classification of these nerves (i.e., as cholinergic, adrenergic, or mixed), and for a proper interpretation of the action of autonomic drugs on the colon. 2. Very little evidence concerning the region of the colon which responds to stimulation of a given nerve, or the direction of the response (circular, longitudinal, or both) is to be found. 3. The pelvic nerves reach the colon deep in the pelvis, but the pathway by which pelvic nerve impulses influence upper levels of the colon is unknown. 4. It is stated (2) that pelvic nerve stimulation causes rapid contraction of the urinary bladder followed by slow relaxation, and it is claimed that atropine does not affect the height of this response but appreciably shortens the period of relaxation. It is unknown whether a similar situation exists in the colon.

The purpose of the present investigation was to supply evidence on these aspects of the motor innervation of the colon.

Methods. Dogs, pigs and monkeys have been used in the present studies. All animals were anesthetized. Sodium pentobarbital has been the principal anesthetic agent, but ether has been employed in certain instances. The pelvic nerves, the hypogastric nerves (both central and peripheral), the vagus nerves, and the coeliac root of the inferior mesenteric ganglia have all been separately subjected to simulation. The stimulus was delivered to the nerve via fixed, shielded electrodes employing a Harvard inductorium and two 4 volt dry cells as the source of current. The inductorium was set at 4.5 to 8.0 cm., and the stimulus was continued for periods of 2 sec. to 2 min. Impulses were delivered at the rate of 60/sec.

The response of the colon to stimulation of various nerves has been recorded by two mechanical systems. The first, the "balloon-enterograph" system was employed to differentiate activity of a circular from that of a longitudinal nature. This system consisted of a balloon inside the colon, and the movable

arm of a Wiggers myocardiograph sewed to the outside of the colon. The myocardiograph was fixed to a ringstand so that changes in this system are a measurement of longitudinal movement of the colon into and out of the pelvis. A water manometer was employed with the balloon system, and a Becker tambour was used with the enterograph.

The second system was a multiple balloon system, and was used to determine which portions of the colon respond to stimulation of a given nerve, and whether or not progressive involvement of the colon existed. One balloon was placed in the proximal colon, one in the middle and one in the distal colon. Each balloon was attached to a water manometer.

In addition to a sample tracing illustrating any given point, a technique has been devised for measuring the extent of a given response of the colon in quantitative terms. This technique involves the measurement, by means of a planimeter, of the area (sq. mm.) enclosed beneath a given response on the kymograph tracing. This area (sq. mm.) divided by the length of the base line over which the response took place (mm.) gives the average height of the response (mm.). The actual measurement of the average height and the duration of a response provides the essential requirements for a comparison of separate responses in a single animal or for obtaining the average response of a series of animals.

In order to determine for a given response the nature of the chemical mediator of the nerve impulse, various autonomic drugs have been employed. These drugs were the mimetic agents acetyl choline bromide (0.01 mgm./K) and epinephrine hydrochloride (0.008–0.011 mgm./K); the blocking agents ergotamine tartrate (0.02–0.03 mgm./K) and atropine sulfate (0.44 mgm./K); and the potentiating agents physostigmine salicylate (0.5 mgm./K) and cocaine hydrochloride (5.0 mgm./K). The acetyl choline and the epinephrine were injected into the aorta just above the origin of the inferior mesenteric artery, and their effect on the colon was noted. The atropine and ergotamine were injected intravenously, and the physostigmine and cocaine were injected subcutaneously. Nerve stimulation of constant strength and duration was conducted before and after the administration of the various blocking and potentiating drugs.

In the studies on the pathway by which pelvic nerve impulses influence upper levels of the colon the following procedures were employed. Two balloons were placed in the colon, one in the distal and one in the descending colon. In one series of experiments the colon was transected between the balloons, and then reanastomosed. In the other series the wall of the colon between the balloons was infiltrated with 2 per cent cocaine.

Results. 1. *Pelvic nerve.* Stimulation of the pelvic nerve caused a contraction of the colon of the dog (37 out of 41 animals); monkey (3 out of 4 animals); and the pig (4 out of 5 animals). Visual inspection of the colon during the course of pelvic nerve stimulation revealed a rather marked longitudinal shortening of the colon. This longitudinal contraction appeared to originate at the most distal end of the colon and gradually involved higher and higher seg-

ments. The net result was a progressive drawing down of the colon into the pelvis. Coincident with the shortening of the colon it appeared to decrease in diameter and became very firm to the touch. A balloon lying free in the distal colon was expelled by this action.

By means of the "balloon-enterograph" system it was observed that this contraction of the colon occurred in both a longitudinal and a circular direction (table 1). However, the relative degree of involvement of the two muscular coats was not constant. Inspection of the mechanograms of this response (fig. 1, A) revealed that it consisted of a rapid contraction followed by a somewhat slower relaxation phase.

Employing the multiple balloon system it was observed (table 2) that in 10 out of 11 dogs the response was confined to the descending and distal colon. In the one remaining animal a rather large response of the proximal colon was noted. The average figures presented in table 2 are, therefore, somewhat misleading. A typical tracing of this response is shown in figure 1, B-1. It can be seen that the response was greatest in the distal colon.

TABLE 1

Type of response of the colon to stimulation of the various nerves

NERVE STIMULATED	NO DOGS	STIMULATION			RESPONSE			
					Circular		Longitudinal	
		No	Ave. strength	Ave. duration	Ave. height	Ave. dura.	Ave. height	Ave. dura.
			cm.	*sec.*	*mm.*	*min.*	*mm.*	*min.*
Pelvic	28	61	7.0	7.5	6.3	2.40	6.1	2.05
Hypogastric	15	34	4.7	26.6	5.6	4.55	−0.3*	0.09
Coeliac root of inf. mes. ganglia	10	29	5.0	30.0	7.7	3.84	−0.5*	0.92

* Indicates movement of the colon out of the pelvis in a few animals.

In a series of 5 dogs it was observed that the response of the descending, but not the distal colon was abolished by transection between these two portions of the colon. The response of the descending colon was not reconstituted by anastomosis of the severed ends of the colon. In order to determine whether the abolition of the response of the descending colon was due to the removal of a mechanical or a nervous influence, 2 per cent cocaine was infiltrated into the wall of the colon between the balloons. In a series of 4 dogs it was observed that such a procedure abolished the response of the descending but not the distal colon. If adequate time was allowed for the cocaine to be absorbed and removed then the response of the descending colon again occurred (fig. 1, C).

In a series of 10 dogs it was shown that physostigmine potentiated the response of the colon to pelvic nerve stimulation (table 3-2). If a constant stimulus was applied to the pelvic nerve before and 10–15 min. after the subcutaneous injection of physostigmine it was possible to show potentiation of the response of the colon to pelvic nerve stimulation without any direct stimulation of the colon by the drug itself (fig. 1, D). If more than 15 min. elapsed follow-

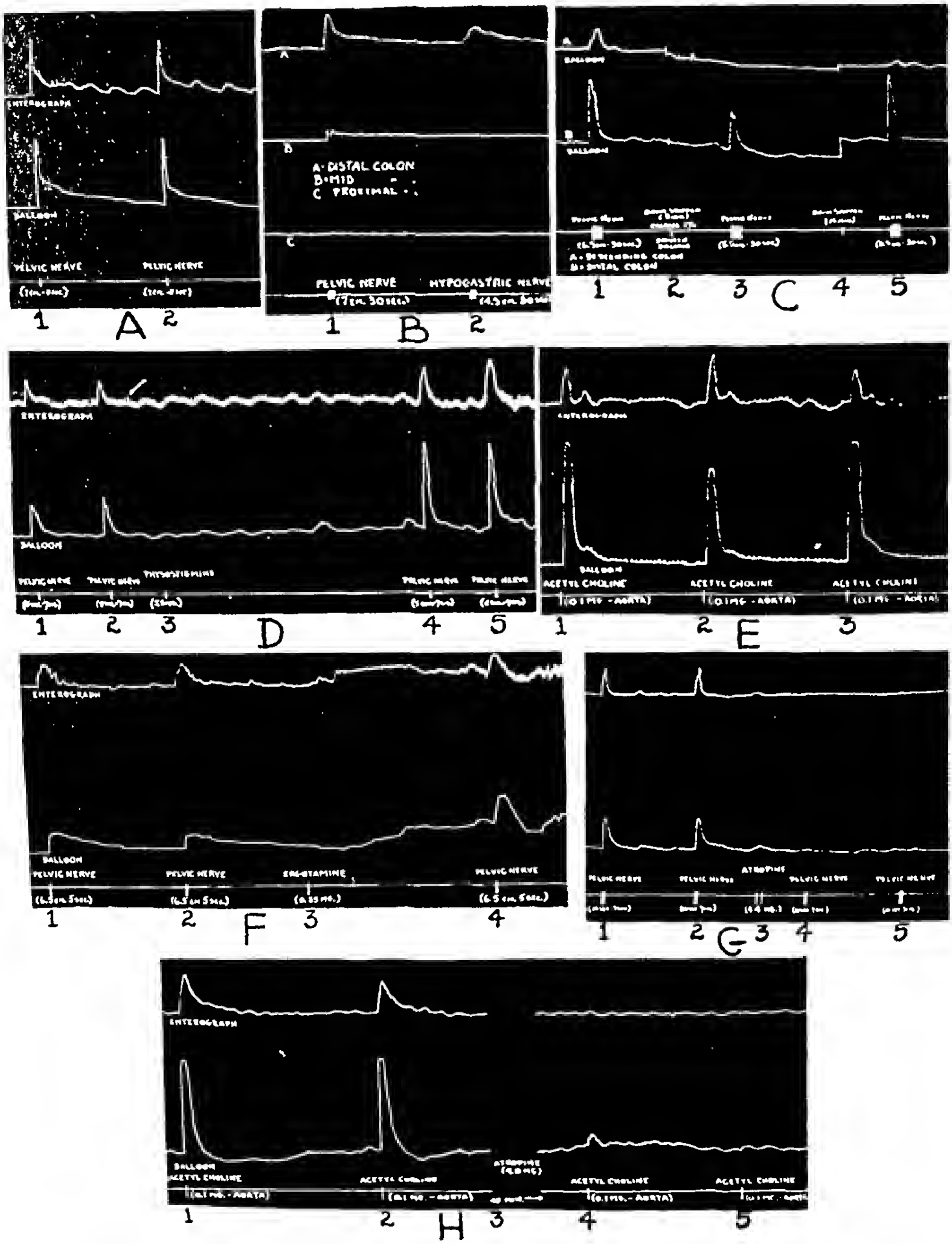

Fig. 1. A (1, 2) pelvic n. stim.; upper, long.; lower, circ.

B (1) pelvic n. stim. (2) hypogastric n. stim.; in descending order balloons in dist. colon, mid. colon, prox. colon.

C (1, 3, 5) pelvic n. stim.; (2) cocaine in wall of colon; (4) drum stopped 15 min. upper line, balloon in descend. colon; lower line, balloon in dist. colon.

D (1, 2, 4, 5) pelvic n. stim.; (3) subcut. physostigmine; upper, long.; lower, circ.

E (1, 2, 3) intra-art. acetyl choline; upper, long.; lower, circu.

F (1, 2, 4) pelvic n. stim.; (3) intraven. ergotamine; upper, long.; lower, circ.

G (1, 2, 4, 5) pelvic n. stim.; (3) intraven. atropine; upper, long.; lower, circ.

ing the injection there was an increase in circular and longitudinal activity of the colon due to the physostigmine.

The intra-arterial injection of acetyl choline was shown in 10 dogs to cause both circular and longitudinal contraction of the colon (table 3-3). The response was similar to that obtained as a result of pelvic nerve stimulation. If the acetyl choline was injected into the aorta just above the origin of the inferior mesenteric artery uniform results were obtained (fig. 1, E). It was apparent that the circular contraction of the colon was somewhat greater following acetyl choline injection than following pelvic nerve stimulation, and the longitudinal contraction was somewhat less.

In 3 dogs the effect of ergotamine on the response of the colon to pelvic nerve stimulation was determined. It was found (fig. 1, F) that ergotamine is without appreciable effect on this response.

It was apparent in 18 dogs that atropine inhibited the response of the colon to pelvic nerve stimulation (table 3-1). If a constant stimulus was applied to the pelvic nerve before and after the intravenous injection of atropine, marked

TABLE 2

Location of the response of the colon to stimulation of various nerves

NERVE STIMULATED	NO. DOGS	STIMULATION			RESPONSE AREA OF THE CONTRACTION CURVE (SQ. CM.)		
		No.	Strength (ave.)	Duration (ave.)	Proximal colon	Descending colon	Distal colon
			cm.	*sec.*			
Pelvic	11	24	6.5	15	0.32*	0.71	2.67
Hypogastric	7	20	4.4	30	0.00	0.00	0.97
Coeliac root of the inf. mes. ganglia	10	28	5.0	30	0.00	3.70	0.00

* Indicates large involvement of proximal colon in only one animal.

inhibition of the response occurred (fig. 1, G). In 7 out of 18 dogs the present dose of atropine completely abolished the response of the colon to pelvic nerve stimulation. In 1 dog the longitudinal response was abolished but some circular action persisted, and in 1 dog the circular response was abolished but some longitudinal action persisted. In the remaining 9 dogs there was a residue of both types of action following atropine. The total per cent inhibition of longitudinal and circular activity was the same.

In those animals in which a residual response remained after 0.44 mgm./K. of atropine, it was observed that multiples of this dose were ineffective in bringing about abolition of the residue. It was observed in 10 dogs that atropine was very effective in inhibiting the response of the colon to the intra-arterial injection of acetyl choline (table 3-3b). If the same dose of acetyl choline was injected before and after atropine, it was apparent that the response was inhibited by atropine (fig. 1, H). In 3 dogs both circular and longitudinal responses were abolished by atropine; in 2 dogs only the circular; in 3 dogs only

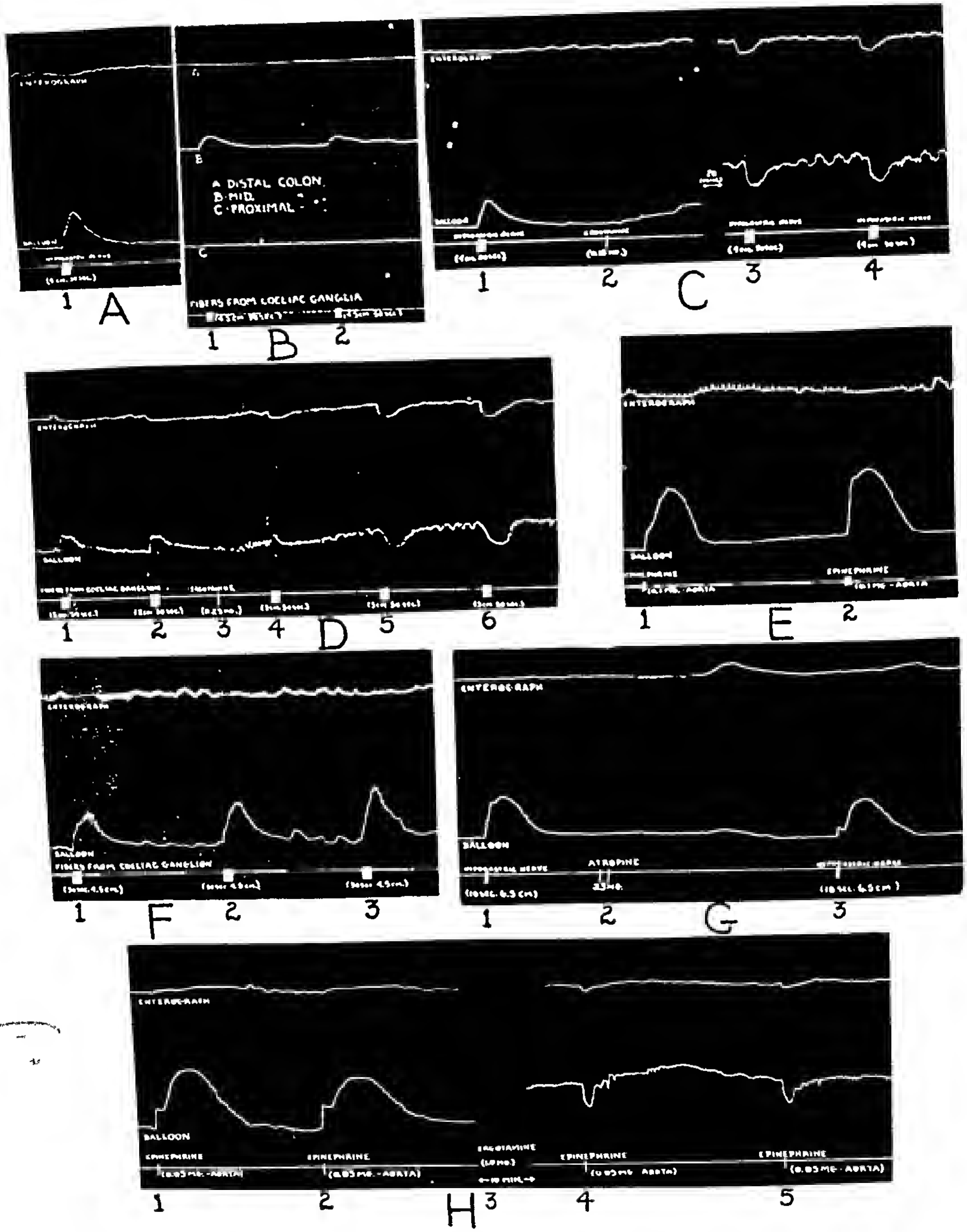

Fig. 2. A (1) hypogastric n. stim.; upper, long.; lower, circ.

B (1, 2) stim. of fibers to coeliac gang. In descend. order, balloons in dist. colon, mid. colon, prox. colon.

C (1, 3, 4) hypogastric n. stim. (2) intraven. ergotamine; upper, long.; lower, circ.

D (1, 2, 4, 5, 6) stim. of fibers to coeliac gang. (3) intraven. ergotamine; upper, long.; lower, circ.

E (1, 2) intra-art. epinephrine; upper, long.; lower, circ.

F (1, 2, 3) stim. of fibers to coeliac gang.; upper, long.; lower, circ.

G (1, 3) hypogastric n. stim. (2) intraven. atropine; upper, long.; lower, circ.

H (1, 2, 4, 5) intra-art. epinephrine (3) intraven. ergotamine, followed by a 10-minute interval; upper, long.; lower, circ.

The effect of atropine on the response of the colon to hypogastric nerve stimulation was determined in 4 dogs. It was apparent that the present dose of atropine did not inhibit the response (table 3-7). If a constant stimulus was applied to the hypogastric nerve before and after atropine, it was observed that there was no appreciable change in the response (fig. 2, G).

Stimulation of the central end of the cut hypogastric nerve was ineffective in producing a response of the colon in a series of 10 dogs.

4. *Coeliac root of the inferior mesenteric ganglia.* Stimulation of those fibers which connect the upper collateral ganglia with the inferior mesenteric ganglia usually produced a response of the colon (15 out of 20 dogs). On visual inspection of the colon this response, like that to hypogastric nerve stimulation, consisted of one or more isolated and stationary rings of constriction.

By means of the multiple balloon system it was observed in 10 dogs that this contraction was confined to the descending colon (table 2). There was no involvement of either the proximal or the distal colon (fig. 2, B).

Employing the "balloon-enterograph" system this response was recorded in 10 dogs (table 1). It was apparent that a circular contraction occurred in all animals. In 1 out of 10 dogs there was depression of the longitudinal recorder indicating movement of the colon out of the pelvis. In general, however, the response consisted of a simple circular contraction of the colon with no change in the longitudinal direction (fig. 2, F).

The effect of ergotamine on the response of the colon to stimulation of the coeliac root of the inferior mesenteric ganglia was determined in a series of 6 dogs (table 3-8). In 3 out of the 6 animals the circular contraction became a relaxation following ergotamine; in 1 animal the contraction was abolished; and in 2 animals it was only inhibited. In the 1 animal which showed depression of the longitudinal recorder before ergotamine, after ergotamine the movement out of the pelvis was increased (fig. 2, D). In another animal in which there was no longitudinal activity before ergotamine, after ergotamine there was movement of the colon out of the pelvis.

It has been shown in experiments on 3 dogs that atropine was without appreciable effect on the present response.

Discussion. The pelvic nerves to the musculature of the colon appear to be cholinergic, as evidenced by the observations that, in general, the response of the colon to pelvic nerve stimulation is inhibited by atropine; potentiated by physostigmine; not inhibited by ergotamine; and mimicked by the injection of acetyl choline.

It is apparent from the present evidence that the response of the colon to pelvic nerve stimulation differs in part from the response to injected acetyl choline. This difference lies in the fact that the injected acetyl choline produces a greater circular response of the colon but a lesser longitudinal response than that produced by pelvic nerve stimulation. It is possible that this discrepancy could be explained by an unequal distribution of nerve fibers to the two muscle layers. If more nerve fibers pass to longitudinal than to circular muscle fibers, then the ratio of longitudinal to circular activity would be high following pelvic nerve stimulation due to a higher concentration of acetyl choline in the longitu-

the longitudinal; and in 1 dog neither longitudinal nor circular responses were completely abolished by this dose. It was apparent (table 3) that there was a 97.5 per cent inhibition of the response of the colon to injected acetyl choline by 0.44 mgm./K. of atropine, but only an 88.6 per cent inhibition of the response to pelvic nerve stimulation by the same dose of atropine.

TABLE 3

The effect of various autonomic drugs on the response of the colon

PROCEDURE	STIMULATION: No.	STIMULATION: Ave. strength	STIMULATION: Ave. duration	DOSE	ROUTE	SERIES	NO. DOGS	RESPONSE: Circular: Ave. height	RESPONSE: Circular: Ave. duration	RESPONSE: Longitudinal: Ave. height	RESPONSE: Longitudinal: Ave. duration	PER CENT CHANGE*: Circular	PER CENT CHANGE*: Longitudinal
		cm.	*sec.*	*mgm. per kgm.*				*mm.*	*min.*	*mm.*	*min.*		
1. Pelvic nerve stim.	78	7.0	10.0			A. Control B. Atropine†	18	6.2 1.7	2.82 1.14	5.6 1.9	2.26 0.79	−88.9	−88.2
2. Pelvic nerve stim.	44	7.0	5.0			A. Control B. Physostig.†	10	6.4 10.9	1.67 2.31	7.1 12.6	1.68 2.15	+135.7	+127.3
3. Acetyl choline inj.	42			0.01	Intra-arterial	A. Control B. Atropine†	10	10.2 1.0	3.30 0.90	3.2 0.6	2.40 0.30	−97.3	−97.6
4. Hypogastric nerve stim.	32	4.7	30.0			A. Control B. Ergotam.†	7	5.3 −2.5	4.74 1.85	−0.7‡ −1.6‡	0.21 0.42	−118.3	+356.2
5. Hypogastric nerve stim.	16	4.5	25.0			A. Control B. Cocaine†	4	4.0 4.3	4.66 5.57	0.0 0.0	0.00 0.00	+28.3	
6. Epinephrine injection	42			0.08–0.11	Intra-arterial	A. Control B. Ergotm.†	10	11.1 −3.7	6.39 4.49	−0.8‡ −3.9‡	0.89 3.19	−123.4	+1643.2
7. Hypogastric nerve stim.	20	5.0	25.0			A. Control B. Atropine†	4	7.6 13.6	6.11 8.13	0.0 0.0	0.00 0.00	+137.9	
8. Stim. of the coeliac root of the inf. mes. ganglia	58	5.4	30.0			A. Control B. Ergotam.†	6	7.0 −3.1	3.60 1.29	−0.4‡ −1.2‡	0.93 0.72	−115.8	+132.8

* Change in the average area of the response curve. Ave. ht. × (Ave. Duration/0.8) (1 min. = 0.8 cm. on kymograph drum).

† After.

‡ Indicates movement of the colon out of the pelvis.

It is evident from table 3 that atropine produced inhibition of both the average height and the duration of the response of the colon to pelvic nerve stimulation.

2. *Vagus nerve.* Stimulation of the vagus nerve, both in the neck and on the esophagus just above the diaphragm, was uniformly ineffective in producing a response of the colon of the dog (10 animals). A slight but inconstant contraction of a portion of the cecum was noted in 1 out of 5 pigs, and in 1 out of 4 monkeys. In the remainder of these latter animals no response of the colon to

vagus nerve stimulation was noted. It was not possible to record the contraction in the pig and monkey colon by balloon techniques. The response appeared to be confined to a portion of the taenia, and slight approximation of silk ligatures tied to the taenia was observed.

3. *Hypogastric nerve.* Stimulation of the peripheral hypogastric nerve produced a response of the colon in only 15 out of 32 dogs. However, when a response was observed it was purely motor. On visual inspection, the response consisted of one or more isolated and stationary rings of constriction. There was no apparent movement of the colon in a longitudinal direction.

By means of the "balloon-enterograph" system it was observed that the contraction of the colon following hypogastric nerve stimulation was primarily circular in nature (table 1). In 2 out of 15 animals there was slight depression of the longitudinal recorder, indicating a movement of the colon out of the pelvis. In general, however, the response was a simple circular contraction, which developed rather slowly and regressed rather slowly (fig. 2, A).

Employing the multiple balloon system it was observed in 7 dogs (table 2) that the response of the colon to hypogastric nerve stimulation was confined to the distal colon. Unlike the response to pelvic nerve stimulation there was no response of the descending colon (fig. 1, B-2).

It was shown in 7 dogs that ergotamine tartrate was effective in inhibiting the circular contraction of the colon in response to hypogastric nerve stimulation (table 3-4). In 2 dogs the response was abolished. In the remaining 5 dogs there was reversal of the response, and after ergotamine a circular relaxation occurred (fig. 2, C). In 1 animal in this series there was a depression of the longitudinal recorder before ergotamine. After ergotamine in this animal the depression was augmented, indicating an increased movement of the colon out of the pelvis. In another animal in this series (fig. 2, C), whereas there was no longitudinal activity before ergotamine, after ergotamine there was movement of the colon out of the pelvis.

The effect on the colon of the intra-arterial injection of epinephrine was determined in 10 dogs. It was shown (table 3-6) that epinephrine caused a circular contraction in all cases. In 2 out of the 10 dogs there was a depression of the longitudinal recorder indicating a movement of the colon out of the pelvis. In the remaining 8 dogs there was a circular contraction of the colon with no appreciable longitudinal response (fig. 2, E). The effect of ergotamine on this response was determined on the above animals (table 3-6). In all 10 animals the circular contraction was reversed and appeared as a relaxation. In the 2 animals in which previous to ergotamine there had been a longitudinal movement of the colon out of the pelvis, this movement was increased following ergotamine. In 6 of the remaining animals in which no longitudinal action was noted before ergotamine, after ergotamine there was a slight movement of the colon out of the pelvis (fig. 2, H).

The effect of cocaine on the response of the colon to hypogastric nerve stimulation was determined in 4 dogs. It was observed that there was slight potentiation of the circular contraction (table 3-5).

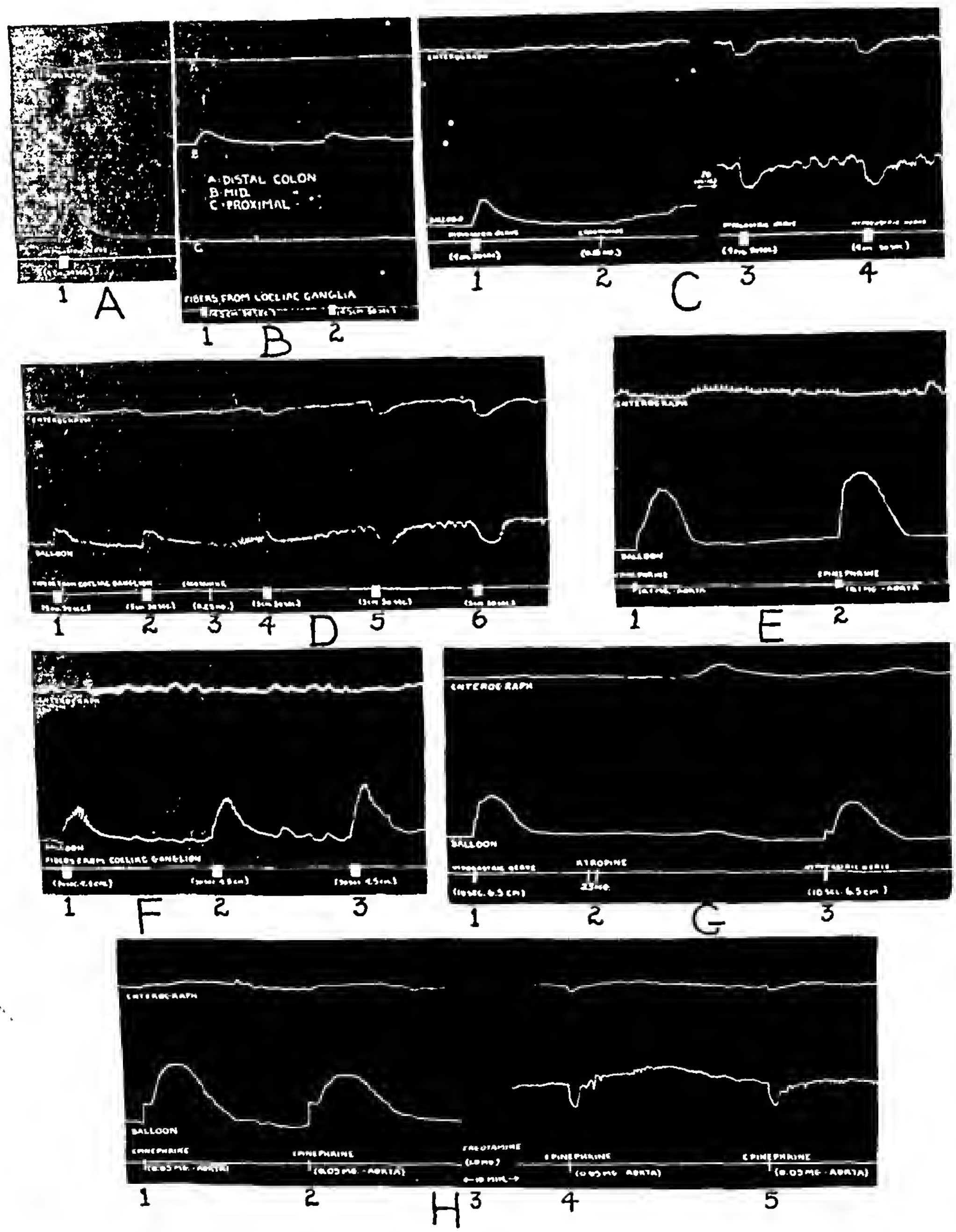

Fig. 2. A (1) hypogastric n. stim.; upper, long.; lower, circ.

B (1, 2) stim. of fibers to coeliac gang. In descend. order, balloons in dist. colon, mid. colon, prox. colon.

C (1, 3, 4) hypogastric n. stim. (2) intraven. ergotamine; upper, long.; lower, circ.

D (1, 2, 4, 5, 6) stim. of fibers to coeliac gang. (3) intraven. ergotamine; upper, long.; lower, circ.

E (1, 2) intra-art. epinephrine; upper, long.; lower, circ.

F (1, 2, 3) stim. of fibers to coeliac gang.; upper, long.; lower, circ.

G (1, 3) hypogastric n. stim. (2) intraven. atropine; upper, long.; lower, circ.

H (1, 2, 4, 5) intra-art. epinephrine (3) intraven. ergotamine, followed by a 10-minute interval; upper, long.; lower, circ.

The effect of atropine on the response of the colon to hypogastric nerve stimulation was determined in 4 dogs. It was apparent that the present dose of atropine did not inhibit the response (table 3-7). If a constant stimulus was applied to the hypogastric nerve before and after atropine, it was observed that there was no appreciable change in the response (fig. 2, G).

Stimulation of the central end of the cut hypogastric nerve was ineffective in producing a response of the colon in a series of 10 dogs.

4. *Coeliac root of the inferior mesenteric ganglia.* Stimulation of those fibers which connect the upper collateral ganglia with the inferior mesenteric ganglia usually produced a response of the colon (15 out of 20 dogs). On visual inspection of the colon this response, like that to hypogastric nerve stimulation, consisted of one or more isolated and stationary rings of constriction.

By means of the multiple balloon system it was observed in 10 dogs that this contraction was confined to the descending colon (table 2). There was no involvement of either the proximal or the distal colon (fig. 2, B).

Employing the "balloon-enterograph" system this response was recorded in 10 dogs (table 1). It was apparent that a circular contraction occurred in all animals. In 1 out of 10 dogs there was depression of the longitudinal recorder indicating movement of the colon out of the pelvis. In general, however, the response consisted of a simple circular contraction of the colon with no change in the longitudinal direction (fig. 2, F).

The effect of ergotamine on the response of the colon to stimulation of the coeliac root of the inferior mesenteric ganglia was determined in a series of 6 dogs (table 3-8). In 3 out of the 6 animals the circular contraction became a relaxation following ergotamine; in 1 animal the contraction was abolished; and in 2 animals it was only inhibited. In the 1 animal which showed depression of the longitudinal recorder before ergotamine, after ergotamine the movement out of the pelvis was increased (fig. 2, D). In another animal in which there was no longitudinal activity before ergotamine, after ergotamine there was movement of the colon out of the pelvis.

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Discussion. The pelvic nerves to the musculature of the colon appear to be cholinergic, as evidenced by the observations that, in general, the response of the colon to pelvic nerve stimulation is inhibited by atropine; potentiated by physostigmine; not inhibited by ergotamine; and mimicked by the injection of acetyl choline.

It is apparent from the present evidence that the response of the colon to pelvic nerve stimulation differs in part from the response to injected acetyl choline. This difference lies in the fact that the injected acetyl choline produces a greater circular response of the colon but a lesser longitudinal response than that produced by pelvic nerve stimulation. It is possible that this discrepancy could be explained by an unequal distribution of nerve fibers to the two muscle layers. If more nerve fibers pass to longitudinal than to circular muscle fibers, then the ratio of longitudinal to circular activity would be high following pelvic nerve stimulation due to a higher concentration of acetyl choline in the longitu-

dinal layer. On the other hand, the injected acetyl choline would have equal access to both muscle groups. This notion assumes that the increased circular response to injected acetyl choline is due to a higher effective concentration of acetyl choline than that produced by pelvic nerve stimulation.

In the present studies it has also been observed that atropine is more effective in inhibiting the response to injected acetyl choline than it is in inhibiting the response to pelvic nerve stimulation. The evidence of Hill (3) indicates that autonomic nerves may enter into smooth muscle cells. If this is true then the present phenomenon may be explained by the theory of Dale and Gaddum (4). This theory states that atropine acts only at cell surfaces and will prevent the action of acetyl choline which must enter the cell, but is without effect upon the action of acetyl choline which is liberated within the cell. If it is assumed that not all the muscle fibers of the colon are directly innervated and that many of them respond to acetyl choline which diffuses from the innervated cells, then it is apparent that atropine would prevent the response of the noninnervated cells, but would not completely abolish the response to pelvic nerve stimulation.

It has been suggested that pelvic nerve fibers extend upward in the hypogastric nerves (5, 6). It might appear that this was the pathway by which pelvic nerve impulses reach the upper portion of the colon. However, in view of the present evidence 1, that stimulation of the central end of the cut hypogastric nerves is without effect on the colon, and 2, that the response of the descending but not the distal colon is abolished by transection or cocainization between these two portions of the colon, it would appear that the most important route of such impulses lies in the wall of the colon.

Henderson and Roepke (2) claim that in the urinary bladder pelvic nerve stimulation causes a dual contractile and tonus response. They state that only the tonus or slow relaxation phase of this response is affected by atropine. The present observation that both the average height and the duration of the response of the colon to pelvic nerve stimulation are inhibited by atropine would indicate that a similar mechanism does not exist in the colon.

The notion that the sympathetic nerves may cause contraction of the colon is not a new one. It has been claimed to occur at times by others (7-10). However, the possibility that epinephrine could cause contraction of the colon was not investigated prior to the studies of Templeton and Lawson (11). The present observations on the motor action of epinephrine on the colon, as part of the proof of the adrenergic nature of the motor effect of the sympathetic nerves on the colon are in general agreement with those of Templeton and Lawson. However, in the present study the secondary increase in longitudinal activity of the colon noted by these investigators following the injection of epinephrine has not been observed.

The explanation for the movement of the colon out of the pelvis occasionally observed following sympathetic nerve stimulation is not apparent. It is unknown whether this movement is due to a longitudinal relaxation below the recorder or a longitudinal contraction above the recorder. However, the abrupt nature of the response suggests that longitudinal contraction occurs above the recorder.

This movement was not abolished by ergotamine. In fact, it was potentiated by this drug. However, this finding does not imply a cholinergic component in the hypogastric nerves, in view of the observation that similar responses were noted following the injection of epinephrine. This longitudinal movement is not explained on the basis of an artifact due to rather strong circular constriction of the colon, as it was observed that reversal of the circular response by ergotamine did not lessen this longitudinal action.

CONCLUSIONS

1. The pelvic nerves to the musculature of the colon are cholinergic. It is suggested that unequal distribution of pelvic nerve fibers to the muscle layers, and the intracellular ending of certain pelvic nerve fibers explain the discrepancies between the response of the colon to pelvic nerve stimulation and that to the injection of acetyl choline. Electrical stimulation of the pelvic nerves causes longitudinal and circular contraction of the descending and distal colon. These nerves influence upper levels of the colon via nerve pathways located in the wall of the colon. A dual contractile and tonus mechanism in response to pelvic nerve stimulation does not exist in the colon as it has been claimed to exist in the urinary bladder.
2. Electrical stimulation of the vagus nerve is ineffective in producing a response of the colon of the dog. However, in certain instances it may produce a weak and inconstant contraction of a portion of the cecum in the pig and monkey.
3. The hypogastric nerves to the musculature of the colon are adrenergic. Electrical stimulation causes a circular contraction which is confined to the distal colon. This effect, however, is not uniformly obtained.
4. The fibers of the coeliac root of the inferior mesenteric ganglia which act on the colon are adrenergic. When they are stimulated electrically a circular contraction which is confined to the descending colon results. This effect, however, is not uniformly obtained.

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RENAL EXCRETION OF POTASSIUM SALTS[1,2]

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The renal excretion of both cations and anions following the injection of various salts of a single typical anion, sulfate, has been the subject of a previous report (13). Certain characteristics of the renal excretion of electrolytes were defined on the basis of these experiments. The present study deals in a similar way with the excretion of cations and anions following the injection of different salts of a single cation, potassium. Any modification of the excretion of potassium could be determined by comparing one experiment with another. Any conditioning of the excretion of anions by potassium could also be studied, at least insofar as data are available through their excretion with cations other than potassium.

METHODS AND MATERIALS. Experiments were conducted with dogs, using multiple successive periods of urine collection and several blood samples before and after injection of potassium salts. The exact procedure has been described elsewhere (13). Creatinine clearance determined in all periods after injection of the salt was assumed to be identical with glomerular filtration rate in the dog (14, 15).

Potassium and sodium were determined by the method of Hald (9), sulfate by the method of Cope (3), phosphate by the method of Fiske and Subbarow (5), chloride by the method of Van Slyke (11). Bromide was determined first by the method of Hastings and Van Dyke (10) and later by the method of Brodie and Friedman (2). pH of urine was determined, using a Hastings compensating colorimeter and suitable indicators (11). With urines of a high pH and a high bicarbonate content the urine was collected directly from the catheter into a glass Ostwald pipette with a stopcock. Bicarbonate of urine was calculated from the total carbon dioxide content of the urine, determined by the method of Van Slyke (11), and the pH of the urine.

RESULTS. In table 1 are presented the data from six experiments in which potassium chloride alone was injected, while in table 2 are the data from five experiments in which potassium bromide alone was injected. In table 3 are recorded the protocols of four experiments in which potassium bromide and neutral potassium phosphate were injected together. Data from two experiments following the injection of potassium sulfate have been presented in detail in a previous paper (13), and so are not reproduced here. These results will be analyzed in various ways.

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[2] A preliminary report of part of this work was presented before the American Physiological Society in 1940: This Journal 129: P498, 1940.

TABLE 1

Intravenous injection of isotonic potassium chloride solution

EXPERIMENT NUMBER	DOG WEIGHT	SALT GIVEN	PERIOD	DURATION	URINE FLOW	CONCENTRATION (M. EQ. PER LITER)						CLEARANCES (CC. PER MINUTE)	
						Urine				Serum			
						K^+	Na^+	Cl^-	HCO_3^-	K^+	Cl^-	K^+	Creatinine
	kgm.	*mM.*		*minutes*	*cc. per minute*								
1	20.5	60.8								3.1	106.3		
			1	59	0.61	26		52				5	
			2*	167	0.66	223		230					
										6.2	106.8		
			3	75	0.36	400		219				26	92
										4.4	107.3		
			4	60	0.22	386		136				18	84
										4.8	106.4		
2	23.2	72.5								3.9	105.7		
			1	18	0.17	41		12				2	
			2*	136	0.88	244		217					
										6.1	108.9		
			3	84	0.36	414		203				25	102
										5.6	109.0		
			4	46	0.22	445		150				18	94
										5.5	107.6		
3†	22.2	77.0								7.2			
			1	33	0.30	86	35	96.6	164			3	
			2*	76	1.26	155	45	148.0	35				
										7.8			
			3	75	0.48	218	10	73.8	101			13	36
										8.1			
			4	120	0.33	189	19	34.9	54			8	30
										6.7			
4	20.7	68.8	1*	78	0.49	243		97	187				
			2	72	0.38	340		106	223				
			3	137	0.13	186		21	290				
			4	1112	0.61			68					
5	24.2	53.6	1*			194		177	77				
			2	50	0.62	311		180	135				
			3	153	1.64	58		28	21				
			4	1100	0.16	79		111					
6	15.0	52.0	1*	75	0.37	243			164	6.6‡			
										6.9‡			
			2	152	0.22	413			325			11	
										9.5‡			
			3	165	0.19	476			63				

* Indicates period during which injection was made.

† NH_4 concentrations in four periods were respectively 190, 16, 11 and 10 mM. per liter urine; PO_4 concentrations were respectively 7, 3, 0 and 10 mM. per liter urine.

‡ Serum pH 7.29, 7.32 and 7.30 respectively determined by Dr. T. Rosenthal using a glass electrode; serum bicarbonate 23.9, 23.4 and 27.4 mM. per liter respectively.

TABLE 2

Intravenous injection of isotonic potassium bromide solution

EXPERIMENT NUMBER	DOG WEIGHT	SALT GIVEN	PERIOD	DURATION	URINE FLOW	CONCENTRATION (M. EQ. PER LITER)										CLEARANCES (CC. PER MINUTE)	
						Urine							Serum			K	Creatinine
						NH_4^+	K^+	Na^+	Cl^-	Br^-	$HPO_4^=$	HCO_3^-	K^+	Cl^-	Br^-		
	kgm.	*mM.*		*minutes*	*cc. per minute*												
7	20.9	74.8											4.4	106.2	0.0		
			1	16	0.47		57		106	0						6	
			2*	133	1.58		128		232	18			7.5	92.2	14.2	23	83
			3	57	0.42		381		231	24			6.5	89.8	14.3	16	72
			4	64	0.31		329		223	19			6.6	89.2	13.9		
8	23.0	80.8											3.2	104.8	0.0		
			1	27	0.17		35		24	0						5	
			2*	51	2.06		250		135	14			5.4		14.6	27	85
			3	70	0.36		433		81	8			6.3		14.1	24	99
			4	65	0.28		497		61	4			5.6	90.3	14.3		
9	17.2	48.7‡											5.2				
			1	44	0.18		59	5	16	0	0	3				2	
			2*	98	0.69	23	101	1	28	3	2	27	8.2			4	30
			3	80	0.31	6	112	0	15	3	0	49	8.9			2	20
			4	122	0.21	2	62	1	16	3	2	23	8.7				
10	21.8	76.5											6.1	110.1	0.0		
			1	12	0.83	13	44	22	48	0	-	14				6	
			2*	83	0.95			75	130	9			11.3	101.3	11.7	22	65
			3	68	0.66	4	324	42	159	11	2	83§	8.0	103.4	11.6	15	56
			4	106	0.37	11	344	21	123	8	46	59§	9.0	101.0	11.2		
11	13.0	57.1											5.2	95.6	0.4	3	
			1	34	0.50	4	37	8	7	0	0	2†					
			2*	74	1.76		163	54	50	6	0	118§	7.5	83.0	14.8	13	42
			3	52	0.52	3	177	4	17	2	0	90§	6.2	84.6	14.2	8	35
			4	118	0.35	8	122	6	9	1	54	17	5.2	86.3	13.7		

* Indicates period during which injection was made.
† Millimols multiplied by 2.
‡ Injected in 500 cc., therefore a hypotonic solution.
§ May be low due to loss of carbon dioxide.

A. *Relationship between urinary excretion of potassium and the concentration of potassium in serum.* In figure 1 excretion rates of potassium are plotted

against corresponding mean concentrations in serum for all experiments, including the two with potassium sulfate previously reported (13). There is obviously

TABLE 3

Intravenous injection of isotonic mixture of potassium bromide and potassium phosphate†

EXPERIMENT NUMBER	DOG WEIGHT	SALT GIVEN	PERIOD	DURATION	URINE FLOW	CONCENTRATION (M EQ. PER LITER)								CLEARANCES (CC. PER MINUTE)	
						Urine				Serum					
						K^+	$HPO_4^{=}$‡	Cl^-	Br^-	K^+	$HPO_4^{=}$‡	Cl^-	Br^-	K	Creatinine
	kgm	*mM.*		*minutes*	*cc per minute*										
12	21.3									4.8	1.6	102.5	0.0		
		KBr . 36.6	1	33	0.11	18	0	9	0					0	
		K_2HPO_4. 16.4	2*	161	0.34	347	238	15	0						
										8.0	4.1		6.8		
		KH_2PO_4 3.8	3	42	0.24	329	247	17	0					11	63
										6.7	3.6	95.6	6.4		
			4	58	0.17	294	254	12	0					8	49
										6.8	3.6	94.6	6.4		
13	21.0									4.2	1.8	100.3	1.8		
			1	10	0.20	60	0	0	0					3	
		KBr 38.5													
		K_2HPO_4. 17.3	2*	90	0.92	267	135	37	1						
										8.2	4.9	92.5	9.0		
		KH_2PO_4. 4.0	3	82	0.46	278	149	44	0					17	51
										6.8	4.0	93.3	8.8		
		Glucose.. 139	4	54	0.35	275	170	26	0					15	55
										6.0	4.3	93.3	8.8		
14	30.3									3.5	1.8	110.5	0.0		
		KBr ... 77.0	1	20	0.10	170	0		0					5	
		K_2HPO_4. . 34.5	2*	122	2.18	165	136		12						
										8.9	4.7	91.6	9.9		
		KH_2PO_4. . .8.1	3	48	0.69	302	151		0					25	104
										7.6	3.6	96.7	8.8		
			4	52	0.46	285	129		0					18	81
										7.1	4.0	96.6	8.3		
			5	70	0.43	300	188		3					19	82
										6.6	4.5	95.0	9.4		
15	13.4									4.5	2.5	107.5	0.0		
		KBr 34.6	1	20	0.15	67	0	7	0					2	
		K_2HPO_4. 15.5	2*	113	0.86	181	109	56	14						
										8.5	5.8	90.8	7.8		
		KH_2PO_4 3.6	3	63	0.95	140	80	13	5					14	84
										9.8	4.0	92.0	9.6		
			4	83	0.32	197	191	16	3					7	58
										8.0	3.6	91.5	7.5		
			5	127	0.24	262	195	8	4					8	55
										8.1	3.2	92.5	7.4		
			6	103	0.19	326	246	5	5					9	52
										6.0	3.1	95.8	7.5		

* Indicates period during which infusion was given.

† The proportion of mono- and dihydrogen phosphates in the mixtures was such that the pH of the mixtures approximated 7.4.

‡ Millimols multiplied by 1.8.

considerable variation among the several experiments in the relationship of excretion rate to serum concentration. Nevertheless there does exist in most

TABLE 2

Intravenous injection of isotonic potassium bromide solution

EXPERIMENT NUMBER	DOG WEIGHT	SALT GIVEN	PERIOD	DURATION	URINE FLOW	CONCENTRATION (M. EQ. PER LITER) Urine NH_4^+	Urine K^+	Urine Na^+	Urine Cl^-	Urine Br^-	Urine $HPO_4^=$	Urine HCO_3^-	Serum K^+	Serum Cl^-	Serum Br^-	CLEARANCES (CC. PER MINUTE) K	Creatinine
	kgm.	*mM.*		*minutes*	*cc. per minute*												
7	20.9	74.8											4.4	106.2	0.0		
			1	16	0.47		57		106	0						6	
			2*	133	1.58		128		232	18							
													7.5	92.2	14.2		
			3	57	0.42		381		231	24						23	83
													6.5	89.8	14.3		
			4	64	0.31		329		223	19						16	72
													6.6	89.2	13.9		
8	23.0	80.8											3.2	104.8	0.0		
			1	27	0.17		35		24	0							
			2*	51	2.06		250		135	14						5	
													5.4		14.6		
			3	70	0.36		433		81	8						27	85
													6.3		14.1		
			4	65	0.28		497		61	4						24	99
													5.6	90.3	14.3		
9	17.2	48.7‡											5.2				
			1	44	0.18		59	5	16	0	0	3				2	
			2*	98	0.69	23	101	1	28	3	2	27				4	30
													8.2				
			3	80	0.31	6	112	0	15	3	0	49				2	20
													8.9				
			4	122	0.21	2	62	1	16	3	2	23					
													8.7				
10	21.8	76.5											6.1	110.1	0.0		
			1	12	0.83	13	44	22	48	0		14				6	
			2*	83	0.95			75	130	9							
													11.3	101.3	11.7		
			3	68	0.66	4	324	42	159	11	2	83§				22	65
													8.0	103.4	11.6		
			4	106	0.37	11	344	21	123	8	46	59§				15	56
													9.0	101.0	11.2		
11	13.0	57.1											5.2	95.6	0.4	3	
			1	34	0.50	4	37	8	7	0	0	2†					
			2*	74	1.76		163	54	50	6	0	118§					
													7.5	83.0	14.8		
			3	52	0.52	3	177	4	17	2	0	90§				13	42
													6.2	84.6	14.2		
			4	118	0.35	8	122	6	9	1	54	17				8	35
													5.2	86.3	13.7		

* Indicates period during which injection was made.
† Millimols multiplied by 2.
‡ Injected in 500 cc., therefore a hypotonic solution.
§ May be low due to loss of carbon dioxide.

A. *Relationship between urinary excretion of potassium and the concentration of potassium in serum.* In figure 1 excretion rates of potassium are plotted

against corresponding mean concentrations in serum for all experiments, including the two with potassium sulfate previously reported (13). There is obviously

TABLE 3

Intravenous injection of isotonic mixture of potassium bromide and potassium phosphate†

EXPERIMENT NUMBER	DOG WEIGHT	SALT GIVEN	PERIOD	DURATION	URINE FLOW	CONCENTRATION (M. EQ. PER LITER) Urine K^+	Urine $HPO_4^{=}$‡	Urine Cl^-	Urine Br^-	Serum K^+	Serum $HPO_4^{=}$‡	Serum Cl^-	Serum Br^-	CLEARANCES (CC. PER MINUTE) K	Creatinine
	kgm.	*mM.*		*minutes*	*cc. per minute*										
12	21.3									4.8	1.6	102.5	0.0		
		KBr . . . 36.6	1	33	0.11	18	0	9	0					0	
		K_2HPO_4. . 16.4	2*	161	0.34	347	238	15	0						
										8.0	4.1		6.8		
		KH_2PO_4. 3.8	3	42	0.24	329	247	17	0					11	63
										6.7	3.6	95.6	6.4		
			4	58	0.17	294	254	12	0					8	49
										6.8	3.6	94.6	6.4		
13	21.0									4.2	1.8	100.3	1.8		
			1	10	0.20	60	0	0	0					3	
		KBr .. 38.5													
		K_2HPO_4. ..17.3	2*	90	0.92	267	135	37	1						
										8.2	4.9	92.5	9.0		
		KH_2PO_4. ..4.0	3	82	0.46	278	149	44	0					17	51
										6.8	4.0	93.3	8.8		
		Glucose.. 139	4	54	0.35	275	170	26	0					15	55
										6.0	4.3	93.3	8.8		
14	30.3									3.5	1.8	110.5	0.0		
		KBr 77.0	1	20	0.10	170	0		0					5	
		K_2HPO_4. ..34.5	2*	122	2.18	165	136		12						
										8.9	4.7	91.6	9.9		
		KH_2PO_4.. ...8.1	3	48	0.69	302	151		0					25	104
										7.6	3.6	96.7	8.8		
			4	52	0.46	285	129		0					18	81
										7.1	4.0	96.6	8.3		
			5	70	0.43	300	188		3					19	82
										6.6	4.5	95.0	9.4		
15	13.4									4.5	2.5	107.5	0.0		
		KBr 34.6	1	20	0.15	67	0	7	0					2	
		K_2HPO_4. .15.5	2*	113	0.86	181	109	56	14						
										8.5	5.8	90.8	7.8		
		KH_2PO_4. ... 3.6	3	63	0.95	140	80	13	5					14	84
										9.8	4.0	92.0	9.6		
			4	83	0.32	197	191	16	3					7	58
										8.0	3.6	91.5	7.5		
			5	127	0.24	262	195	8	4					8	55
										8.1	3.2	92.5	7.4		
			6	103	0.19	326	246	5	5					9	52
										6.0	3.1	95.8	7.5		

* Indicates period during which infusion was given.

† The proportion of mono- and dihydrogen phosphates in the mixtures was such that the pH of the mixtures approximated 7.4.

‡ Millimols multiplied by 1.8.

considerable variation among the several experiments in the relationship of excretion rate to serum concentration. Nevertheless there does exist in most

individual experiments a direct relationship between excretion rate and serum concentration. The lines rise very sharply with slight increase in concentration of potassium in serum. The one exception is the line in the extreme lower right hand corner, corresponding to experiment 9 of table 2. The lines are in general not exactly straight. The upper portions, representing exogenous excretion, usually have a steeper slope than the lower portions, representing the transition between exogenous and endogenous excretion. In other words, extrapolations of the former would, in most instances, cut the abscissa at points slightly above those corresponding to endogenous excretion, and of course well above the origin. There is no definite grouping of the lines according to the salts or salt mixtures injected.

Clearances of potassium vary markedly with the concentration of potassium in serum, being greater the higher the serum concentration (tables 1, 2, 3).

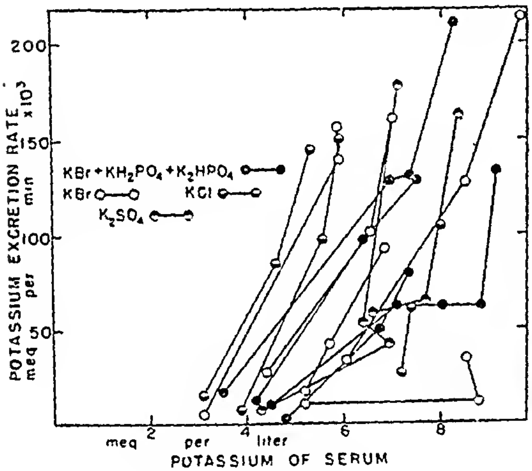

Fig. 1

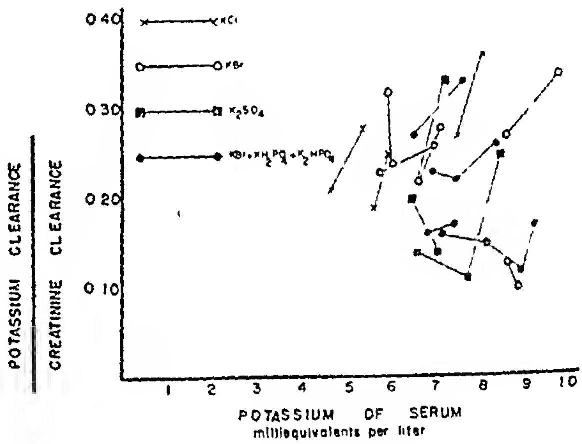

Fig. 2

Fig. 1. Relationship between urinary excretion rate and simultaneous concentration of potassium in serum

Fig. 2. Tubular reabsorption of potassium. Ratio of potassium to creatinine clearance measures the proportion of filtered potassium which escapes reabsorption by the renal tubules.

B. *Relation between potassium excretion and glomerular filtration rate.* In figure 2 the ratio of potassium to simultaneous creatinine clearance is plotted against mean serum concentration. Since the ratio of the clearances falls between the limits 0.10 and 0.40, the two clearances are fairly well correlated; in other words, clearance of potassium varies proportionally with the glomerular filtration rate, except as slight changes in tubular reabsorption perturb this simple relationship.

On the other hand, so considerable is the dispersal of the points in figure 2 that it is difficult to be sure that any correlation at all between the clearance ratio and the concentration of serum potassium exists. The difference between the clearance ratio and unity measures the proportion of filtered potassium reabsorbed by the tubules. This proportion is certainly somewhat greater during all periods of active excretion of injected potassium than during the preliminary

control periods, since a number of points lying close to the abscissa between 4 and 5 would be present if creatinine clearance had been measured in these preliminary periods. There is, however, no evidence from this chart that potassium clearance tends to approach creatinine clearance as serum concentration rises, and it is evident that, within the range of potassium which can be tolerated physiologically, the greater part of the filtered potassium is still being reabsorbed.

C. *Effect of associated anions on potassium excretion.* Sulfate is a rapidly excreted anion with a high clearance (8, 13), chloride and bromide are slowly excreted anions with low clearances (16). Phosphate in this respect lies somewhere in between (12). Nevertheless, the lines in figure 1 corresponding to these four classes of experiments are not arranged in any systematic order corresponding to the salt injected, although potassium excretion corresponding to a given serum concentration varied considerably from experiment to experiment.

The very low excretion rate of potassium in experiment 9 of table 2 (the line lies in the lower right hand corner of fig. 1) is somewhat puzzling. There was no evidence of renal disease in this animal, although creatinine clearances are somewhat low. Perhaps the explanation may be found in the fact that here the potassium bromide was injected in a hypotonic solution, and that in consequence the usual increased renal blood flow and glomerular filtration produced by an infusion of an isotonic salt solution were lacking.

D. *Relation of potassium excretion to that of other ions.* 1. *Effect on the acid base balance of urine and the excretion of bicarbonate.* A most striking effect of the injection of either potassium chloride or of potassium bromide was an immediate enormous increase in the excretion of bicarbonate as soon as the infusion was started (tables 1, 2). This was accompanied by a sharp rise in the pH of the urine, frequently to the extreme upper limits of physiologically tolerable alkalinity (fig. 3). The increased excretion of bicarbonate and the high urinary pH continued for some time after the injection was finished.

No such rise in pH of urine occurred after potassium sulfate injection (fig. 3). The pH of the urine after the infusion of phosphate mixtures was usually fixed for some time close to pH 7.4, the pH of the injected buffer itself.

2. *Effect on the excretion of sodium.* It has previously been shown that the injection of potassium sulfate is followed by a sharp increase in the excretion of sodium, which continues for some time after the end of infusion (13). In one experiment with potassium chloride there was a slight increase of sodium excretion during the infusion itself, followed at once by a fall below the pre-injection rate in the subsequent periods (table 1, expt. 3). The same is true in two of three experiments with potassium bromide (table 2, expts. 10, 11), while in a third (table 2, expt. 9) the rise of sodium excretion during injection is absent.

3. *Effects on the excretion of chloride and bromide.* The injection of potassium bromide is followed by a moderate increase in the excretion of both bromide and chloride, that of potassium chloride by an increased excretion of the latter alone. The total increased excretion of halide is much less than that of potassium.

There is no radical difference between the excretion of halide in these experiments and the excretion of halide following the injections of sodium bromide or of sodium chloride (1, 13). The ratio of chloride to bromide in urine is, as usual, higher in urine than in serum, indicating some preferential reabsorption of the bromide. Otherwise they seem to behave more or less interchangeably.

There is, however, a marked difference between the excretion of halide following the injection of potassium bromide or chloride alone and the excretion of halide following the injection of mixtures of potassium bromide with potassium phosphate. There seems to be a distinct inhibition of halide excretion in the presence of potassium phosphate. This is shown in figure 4, in which the cumulative recovery of halide following injection of pure potassium bromide and potassium chloride is compared with the recovery following injection of the mixtures with potassium phosphate.

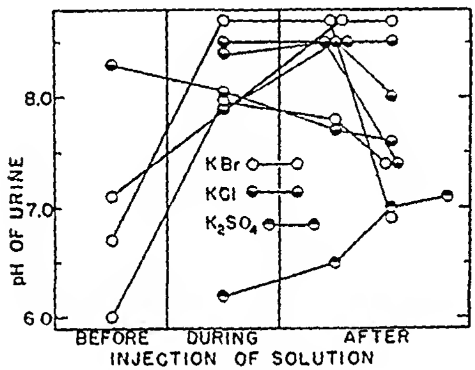

Fig. 3

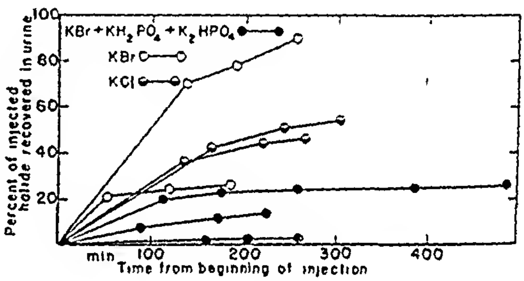

Fig. 4

Fig. 3. Effects of infusions of neutral salt solutions on pH of urine. Injection of potassium chloride or potassium bromide solution is followed by a sharp rise in the pH of urine, while injection of potassium sulfate solution is not.

Fig. 4. Effect of associated anion on the rate of excretion of injected halide. Potassium chloride and bromide are excreted more rapidly when injected alone than when accompanied by potassium phosphate.

4. *Effect on the excretion of phosphate.* No increased excretion of phosphate followed the injection of potassium chloride (table 1, exp. 3) or of potassium bromide (table 2, expts. 9, 10 and 11). Phosphate concentration in serum is elevated and urinary excretion of phosphate increased following the injection of mixtures of potassium phosphate and bromide (table 3). It is difficult to determine whether or not there is anything peculiar in its mode of excretion in these experiments, since few data on the excretion of phosphate with cations other than potassium are available.

5. *Effect on ammonia excretion.* A low rate of excretion of ammonia followed the injection of potassium chloride (table 1, expt. 3) and the injection of potassium bromide (table 2, expts. 9, 10 and 11).

Discussion. The functional relationship between the excretion rate of potassium and its concentration is clearly indicated in figure 1. The behavior of potassium in this respect resembles that of sulfate (13). The behavior of potas-

sium is similar to that of sulfate in at least two other respects. First, the lines relating serum concentration and excretion rate do not pass through the origin (fig. 1). Secondly, the straight lines representing exogenous excretion, when prolonged, do not in general pass through the points representing endogenous excretion. Because the lines do not pass through the origin, it follows mathematically that the clearance of potassium must depend upon serum concentration, being greater the higher the serum concentration. The algebraic formulation of this fact has been fully discussed in connection with the parallel formulation in the case of sulfate excretion (13), and need not be repeated here. This variation of clearance with serum concentration seems to be quite characteristic of many electrolytes. This behavior is, of course, very different from that of many organic filterable solutes, whose representative lines do pass through the origin, so that their clearances are more or less independent of serum concentration. Because the lines representing exogenous excretion do not in general pass through the points representing endogenous excretion, a curve characterizing the whole of potassium excretion cannot be represented by a single straight line. It may be approximated by two lines, one with a steep slope, representing excretion at high concentrations, and another with a lesser slope, representing excretion at low concentrations. Whether the junction of these two lines is gradual or abrupt cannot be determined from our experiments. It is certain, however, that the excretion of potassium is a different function of serum concentration after the latter has been raised by the injection of potassium salts than when it lies within the usual physiological range.

The lines of figure 1 seem to indicate that the excretion of potassium is not dependent on the associated anion. In a sense these lines characterize a specific mode of excretion peculiar to potassium. This at once brings up the fundamental dilemma which confronts the student of electrolyte excretion: how may each individual ion be excreted more or less according to its own individual law, while at the same time the sum of the acid equivalents in urine must always equal the sum of the base equivalents? Our experiments throw considerable light on this problem. When neutral potassium chloride or bromide solutions are injected, bicarbonate excretion in the urine at once increases greatly, so that the urine becomes distinctly alkaline. Bicarbonate content and pH of serum are but little affected (table 1, expt. 6). When neutral potassium sulfate is given, on the other hand, there is a marked and sustained increase in sodium excretion without appreciable change in urinary pH. Halide is characteristically excreted slowly, potassium much more rapidly; bicarbonate is apparently obligingly excreted to make up the difference. Sulfate clearance is considerably greater than that of potassium; each persists in its own stiff-necked way, and sodium is excreted in amounts just sufficient to maintain the electroneutrality of the urine. These reactions serve perfectly to permit potassium, sulfate and halide each to be excreted in its own characteristic manner. In order to do this, of course, bicarbonate and sodium are sacrificed according to the requirements of electroneutrality, rather than according to any individual laws of their own. Gamble has written elsewhere (6) of the "mendicant" rôle of bicar-

bonate in the bodily economy; perhaps we must include sodium in the same category, at least with respect to its rôle in urinary excretion under these peculiar circumstances.

The relative inhibition of halide excretion in those experiments in which potassium phosphate was injected along with potassium bromide may be in some way analogous to the inhibition of chloride excretion when mixtures of sodium chloride and sodium sulfate are injected (13). Certainly the delayed excretion is not simply a reflection of a reduced serum halide consequent upon dilution of the body fluids by the phosphate infusion, since serum halide actually rose in one of the experiments with phosphate mixtures in which halide excretion was much retarded (table 3, expt. 13). Also, in the experiment with the greatest recovery of halide following potassium bromide (table 2, expt. 7), serum halide fell during the course of the experiment.

Potassium and creatinine clearances are closely enough correlated to indicate that the former depends in part on the latter. Figure 2 gives no indication of any upper limit to reabsorption, even though the proportion of filtered potassium reabsorbed by the tubules is somewhat less after potassium injection than under ordinary circumstances. Apparently the kidney eliminates injected potassium very rapidly, partly by an increase in the amount filtered and partly by a moderate reduction in the proportion reabsorbed.

The rather high concentrations of potassium sometimes found in the urine following injections of potassium chloride or bromide are a little surprising. Concentrations greater than 400 mM per liter urine are found in experiments in which isotonic solutions were injected without subjecting the animal to any particular dehydrating procedure. In experiments with injections of hypertonic sodium chloride or sodium sulfate or in experiments with severe water deprivation the concentration of sodium in the urine seldom rises above 350 mM per liter (4, 7). It is unlikely that in such experiments the excretion of sodium is limited by osmotic factors *per se*, since higher osmolar concentrations appear in our experiments in the apparent absence of any obligation to conserve water.

SUMMARY AND CONCLUSIONS

1. Potassium excretion in the urine following injection of potassium salts is directly correlated with serum concentration. It is little affected by the particular associated anions.

2. Potassium excretion is a somewhat different function of serum concentration at high than at low concentrations of potassium in serum.

3. Clearance of potassium varies sharply with its concentration in serum, being greater at higher serum concentrations. It varies in part with glomerular filtration rate, but never approaches the latter in absolute magnitude.

4. Intravenous injection of neutral potassium bromide or potassium chloride is followed by the excretion of an intensely alkaline urine containing large amounts of bicarbonate.

5. Intravenous injection of neutral potassium sulfate produces no change in the reaction of the urine but is followed by a marked increase in sodium excretion.

6. These experiments illustrate one type of physiological reaction which preserves the effective electroneutrality of the urine yet permits potassium, sulfate and halide each to be excreted according to its characteristic law.

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CONTROL OF NORMAL BREATHING IN FISHES BY RECEPTORS LOCATED IN THE REGIONS OF THE GILLS AND INNERVATED BY THE IXTH AND XTH CRANIAL NERVES[1]

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In view of the fact that Heymans, Bouckaert and Dautrebande (1) and Heymans and Bouckaert (2) had found the carotid chemoreceptors sensitive to changes in the carbon dioxide tension of the blood of mammals it was suggested by Powers (3) that the regions of the gills might be the loci of receptors for chemotaxic responses of fishes to the carbon dioxide tension of the water. Instead these chemotaxic receptors were found to be located in the lateral line organs, innervated by a branch of the Xth cranial nerve (4). It was found that fishes with lateral line nerves sectioned just distal to the gills do not respond to the carbon dioxide tension of the water, even though branches of the IXth and Xth cranial nerves leading to the gills are intact. This observation eliminates the regions of the gills as loci of receptors for chemotaxic responses to the carbon dioxide tension of the water.

During this investigation (4) different combinations of sectionings of the IXth and Xth cranial nerves just central to the gills were carried out on the brook trout, *Salvelinus f. fontinalis* (Mitchell), the rainbow trout, *Salmo gairdnerii irideus* Gibbons and the blue gill, *Lepomis macrochirus* Rafinesque. Observations made seemed to substantiate suggestions made by Marshall and Rosenfeld (5) and Schmidt and Comroe (6) that the chemoreceptors "might represent the survival in the air breathing adult, of an organization of utmost significance to the water-breathing forebear." It was found that each of the three species of fishes investigated responded similarly to each combination of sectioning of the IXth and Xth cranial nerves just central to the gills (see table 1). The fishes breathed at approximately the same rate before and after the bilateral sectioning of the Xth nerves only. As a rule the fishes showed a gasping type of breathing before dying (table 1). The responses to the bilateral sectioning of the IXth nerves only were entirely different from the responses to the bilateral sectioning of the Xth nerves only. The rule without exception was the cessation of all breathing which was followed by a gasping type of breathing. When the IXth and Xth nerves were both bilaterally sectioned the responses were similar to the responses when the IXth nerves only were bilaterally sectioned except perhaps intensified. There were only two exceptions. The first two fish, brook trout, operated upon at first breathed approximately normally after the operation except at a slight reduction in rate. It is believed that portions of the IXth nerve were missed on one side. This is borne out by the fact that

[1] Contribution no. 8, Department of Zoology and Entomology, University of Tennessee, Knoxville.

breathing of all fishes was approximately normal when the IXth or Xth or IXth and Xth were sectioned on one side only. These two fishes as well as a second brook trout and a blue gill in which it was known that a part of the IXth nerve was missed are not included in the table. All four fishes responded in the same way.

When table 1 is scrutinized certain outstanding facts are noted. 1. No fish survived an operation for any length of time. Since fishes in which the gills were mutilated died almost if not as quickly as after other operations it seems reasonable to suppose that the rapid death was due to loss of blood. The efferent branchial arteries could not be avoided when the IXth and Xth nerves were sectioned just central to the gills. 2. When the Xth nerves only were bilaterally sectioned the breathing was at first approximately normal. As a rule this was followed by a gasping type of breathing. 3. The bilateral sectioning of the IXth or the IXth and Xth nerves was always followed by a cessation of all breathing. After a few seconds, always less than a minute, this was followed by a gasping type of breathing or in a few exceptions there was a jerky type of breathing. This jerky type of breathing was interpreted as being a weak gasp. 4. When the IXth nerve or even a part of the IXth nerve leading to the gills remained intact on one side the breathing approximated normal. One rainbow trout and one blue gill, not included in the table, in which a part of the IXth nerve was known to have been missed on one side breathed approximately normally for a brief period. A gasping type of breathing was the exception (IXth nerve intact on one side) and not the rule. These observations point to the conclusion that receptors located in the regions of the gills and innervated by the IXth cranial nerves are more important in controlling normal breathing of fishes than the cephalic or central portion of the nervous system. This does not pre-

TABLE 1

The table gives a summary of breathing responses after each operation as indicated. The sectioning of the nerve or nerves indicated was just central to the gills. No fish lived longer than a few minutes after an operation. Approximately normal means that the rate of breathing was approximately the same after as before an operation.

NO.	NAME OF FISH	TYPE OF OPERATION	KIND OF BREATHING AFTER OPERATION
4	Brook trout	Gills mutilated	Approximately normal
3	Rainbow trout	Gills mutilated	Approximately normal
2	Blue gills	Gills mutilated	Approximately normal
6	Brook trout	Xth bilateral	Approximately normal
2	Rainbow trout	Xth bilateral	Approximately normal
4	Brook trout	IXth bilateral	Normal breathing ceased, few gasps
4	Rainbow trout	IXth bilateral	Normal breathing ceased, few gasps
All	Fishes	IXth or Xth unilateral	Approximately normal
4	Brook trout	IXth and Xth unilateral	Approximately normal
4	Brook trout	IXth and Xth bilateral	Normal breathing ceased, few gasps
4	Rainbow trout	IXth and Xth bilateral	Normal breathing ceased, few gasps
4	Blue gills	IXth and Xth bilateral	Normal breathing ceased, few gasps

clude the localization of an "apneustic center" in the cephalic portion of the nervous system (7) but adds evidence to the views holding to the presence of an "apneustic center" and its inhibition by incoming impluses over the IXth and Xth nerves with inhibition by the IXth being more effective than by the Xth.

The apparent inhibition of the IXth might be due to asphyxiation through the loss of blood to the brain (8) but is hardly the case since the mutilation of the gills in which there was at least the same loss of blood did not produce gasping. Neither do the observations preclude the inherent rhythm of the respiratory center suggested by Adrian and Buytendijk (9) for the goldfish. The observations do add evidence to the dominance of impulses through the IXth nerves over all breathing movements. One rainbow trout in which the IXth and Xth nerves were sectioned on both sides died without breathing or gasping. This observation is evidence in support of the views held that breathing rhythm is reflexly excited. The more central the IXth nerves are sectioned the more nearly completely are all of the branches severed. Lutz and Wyman (10), Boyd (11), Herrick (12) and Pearsons (13) have described the location of receptors innervated by the IXth nerves, i.e., the innervation by the IXth nerves. Without entering into the controversy as to the origin, whether central or reflex in mammals, of impulses initiating efferent impulses from the respiratory center (14) it might be stated that the afferent reflex paths are much more restricted in fishes than in mammals due to the less high development of the brain of fishes. Fishes have no cerebral cortex. The only higher center is the rhinencephalon, mainly the nucleus of the first cranial nerves, and the corpora striata. As the brain increases in complexity, other centers as they develop might take over prominent controls of normal breathing and become less segmentally dominated (15). Summaries of current views with literature cited have been presented by Heymans and Bouckaert (2), Gesell (16), Gellhorn and Lambert (17), Pitts (18) and Schmidt and Comroe (6). Observations reported in this paper indicate that normal breathing in fishes is dominantly reflex, originating in receptors located in the regions of the gills and innervated by the IXth cranial nerves.

Acknowledgments. The authors wish to thank Mr. E. L. Green, Assistant Wildlife Technician, and Mr. J. R. Eakin, Superintendent, The Great Smoky Mountains National Park, for coöperating in making fishes available without which the experiments here reported could not have been carried out.

SUMMARY

1. Different combinations of sectionings of the IXth and Xth cranial nerves just central to the gills were carried out in three species of fishes.

2. The three species of fishes responded similarly to each type of sectioning.

3. When the Xth nerve was unilaterally or bilaterally sectioned breathing continued approximately normal and was often followed by a gasping type of breathing before death.

4. When the IXth or the IXth and Xth nerves were unilaterally sectioned breathing continued approximately normal. Death followed generally without gasping.

5. When the IXth or the IXth and Xth nerves were bilaterally sectioned there was always cessation of all breathing. As a rule this was followed by a gasping type of breathing.

6. From these observations it was concluded that the control of normal breathing in fishes is dominantly reflex originating in receptors located in the regions of the gills and innervated by the IXth cranial nerves.

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THE DISTRIBUTION, FLOW, PROTEIN AND UREA CONTENT OF RENAL LYMPH[1]

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Most authors are in agreement concerning the extra-renal pathway of the lymphatics of the mammalian kidney, though there is still disagreement as to the relationship of the lymph vessels of the renal capsule and those within the kidney itself. Thus, Bartels (1), Fuchs and Popper (2) and Abeshouse (3) believe that the capsular lymphatics may participate in the drainage of the kidney parenchyma, while Parker (4) was unable to find any evidence of parenchymally injected dye in the renal capsular lymphatics. With respect to the rate of flow of lymph from the kidney, the only data are those reported by Schmidt and Hayman (5), who measured the rate of lymph flow in the thoracic duct and in the abdominal receptaculum of dogs after preliminary evisceration, unilateral nephrectomy, ligation of aorta and inferior vena cava below the remaining kidney and ligation of the portal vein and hepatic artery. The flow of lymph thus measured was considered indicative of the lymph drainage of the remaining kidney. They observed an increased lymph flow after the administration of various diuretics and of adrenalin. About the chemical composition of renal lymph, nothing is known except for a single protein determination in a sample collected directly from a dog's kidney by Drinker and Field (6).

In the present communication, the results of a study concerning the capsular and hilar lymph drainage of the dog's kidney, together with the protein and urea content of twelve different samples of lymph collected directly from the renal lymph vessels, are presented. These determinations are compared with the protein and urea content of plasma obtained from the renal artery and renal vein at the conclusion of the period of lymph collection.

METHODS. A. *Exposure of the kidney.* Normal dogs, anesthetized with nembutal (pentobarbital sodium) were employed in these experiments. A skin incision was made parallel to and one inch below the last rib on the left side and was extended from the midline to the vertebral column. Successive abdominal muscles were split in the line of their cleavage until the peritoneum was reached. The latter was incised, the posterior muscular portion of the incision bisected, and the superior and inferior flaps were then sutured to the skin of the lower external chest and lower abdomen respectively. This procedure exposed both upper and lower poles of the kidney and with a minimal amount of blunt dissection and manual traction, all surfaces of the kidney could be exposed and visualized.

[1]Aided by a grant from the Dazian Foundation for Medical Research.

B. *Injection of Evans blue dye.* A ten per cent solution of Evans Blue dye was used for all renal parenchymal injections. A 27 gauge needle, attached to a tuberculin syringe, containing the dye, was inserted $\frac{1}{8}$ inch into the renal parenchyma for cortical injection and $\frac{3}{8}$ inch into the parenchyma for medullary injection. Approximately 0.2 to 0.5 cc. of the dye was injected slowly so as not to produce a cystic area within the kidney. A thin stream of water was directed against the injection site, as the needle was withdrawn quickly, to prevent the superficial extravasation of concentrated dye into the capsular tissue.

C. *Cannulation and collection of renal lymph.* The cannulae employed for these collections were made of glass tubing (3 mm. in diameter), one end of which was drawn out to a calibre of approximately 1 mm. in external diameter. A very small amount of dried, purified heparin was placed in the interior of the cannula to prevent clotting.

The lymph vessels of both the capsular and hilar areas of the kidney were searched for, and when visualized, ligated. Those of the capsule were ligated at each pole of the kidney, and those in the hilar area were ligated near the juxta-aortic nodes into which they drained. The preparation was allowed to stand for 20 minutes, during which time the lymph vessels proximal to the ligatures distended easily to twice their previous size. It was noted many times that ligation of the hilar vessels effected a distention in the capsular ones. A loose ligature having been placed about the distended lymph vessel, the cannula was inserted into it distal to the loose ligature and with the preliminary rapid flow of clear fluid into the cannula, the ligature was then tightened about the inserted end of the cannula. Usually 0.5 to 1.0 cc. of lymph could be collected from one of these vessels within 20–40 minutes. It was found that the flow from any single lymph trunk was accentuated by the ligation of the remaining, visible trunks. At the end of the collection, renal arterial and venous blood samples, the femoral artery pressure and the weight of the kidney of the dog were taken.

D. *The determination of the protein and urea content of the lymph and blood plasma samples.* Protein in both lymph and plasma was determined by a modification of Kingsley's biuret method (7). The color was measured in the Evelyn photoelectric colorimeter and the concentration obtained from a calibration curve based on gravimetric protein determinations. Urea was determined by a urease-aeration-titration method (8).

Results. A. *The distribution of Evans blue dye after injection into the medullary area of the kidney.* The injection of Evans Blue dye into the medullary area of 15 different kidneys of normal anesthetized dogs was followed by the appearance of the dye in the hilar lymph vessels of all injected kidneys within 0.5 to 4.5 minutes, whereas it was found in the capsular lymph vessels of only two of the 15 injected kidneys. The hilar lymph vessels were seen to traverse the loose connective tissue surrounding the renal artery and vein, emptying as has been described (4) into the lateral abdominal lymph chain. The number of vessels varied considerably in each injected kidney, usually about six large vessels being seen, some of which were in intimate association with the renal artery and vein.

B. *The distribution of Evans Blue dye after injection into the cortical area of the kidney.* Contrary to the results following medullary injection of the dye, it was found that when injections were made into the cortical area of eleven different kidneys, the dye was observed in the external capsular lymph vessels of eight of the injected kidneys, and in the hilar vessels of seven of them. In five, the dye was observed in both the hilar and the external capsular lymph vessels. The latter lymph trunks were usually two in number, each of which traversed opposite poles of the kidney to continue medially in the perirenal tissue and to end finally in the lateral abdominal lymph chain. Occasionally, a large capsular lymph vessel was observed, leaving the parenchyma of the kidney, and piercing

TABLE 1

The flow, protein and urea content of renal lymph

EXPT. NO.	WEIGHT OF SINGLE KIDNEY	RATE OF FLOW FROM THE CANNULATED LYMPH TRUNK	PROTEIN CONCENTRATIONS			UREA CONCENTRATIONS			LYMPH UREA PER CENT PERCENTAGE CHANGE RELATIVE TO RENAL VEIN PLASMA UREA PER CENT	RENAL VEIN PLASMA UREA PER CENT PERCENTAGE CHANGE RELATIVE TO RENAL ARTERY PLASMA UREA PER CENT
			Renal lymph	Renal artery plasma	Renal vein plasma	Renal lymph	Renal artery plasma	Renal vein plasma		
	gm.	*gm./min.*	*gm. per cent*	*gm. per cent*	*gm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*
1	38.7	0.0054	4.21	5.84	5.92	84.0	47.5	44.0	+91	−6.4
2*	45.7	0.0076	2.80	6.09	5.75	70.4	61.5	58.0	+22	−6.7
3a	—	0.0102	3.00	5.35	5.19	52.1	22.8	22.8	+128	±0.0
4	52.9	0.0114	2.35	5.18	5.18	47.7	26.4	24.6	+94	−6.8
5*	40.4	0.0129	1.83	5.42	5.12	47.7	38.7	35.2	+36	−9.0
6	44.2	0.0153	2.04	5.55	6.35	48.4	44.0	44.0	+10	±0.0
7	56.6	0.0229	0.96	5.60	5.57	58.6	52.8	51.0	+15	−1.5
8*	40.3	0.0270	1.12	6.88	6.52	112.5	82.6	79.1	+42	−4.2
9	64.5	0.0329	0.44	6.45	6.45	164.0	154.6	151.2	+9	−1.2
3b	—	0.0352	1.41	5.35	5.19	42.2	22.8	22.8	+85	±0.0
10	—	0.0425	0.56	6.10	6.60					
11	43.1	0.0550	1.34	5.95	5.81	38.7	30.8	29.9	+33	−3.8
Average......		0.0232	1.84	5.81	5.80	69.7	53.1	51.1	+55	−3.6

* Renal lymph obtained from capsular vessel.

the fibrous capsule to join one of the two large trunks running in the fatty capsule toward either pole. Invariably, ligation of the external capsular lymph trunk was followed by distention of the trunk segment proximal to the ligature, indicating that the flow of lymph was distalward from the kidney.

C. *The distribution of dye following intra-renal artery injection of Evans Blue dye.* Injection of the dye (2.0 cc.) directly into the renal artery of three different kidneys was followed with 0.5 to 1.0 minute by the appearance of the dye in both the external capsular and hilar lymph vessels of each kidney. It was found also that whereas the parenchyma of the kidney (both cortical and medullary areas) was rapidly and uniformly stained with the dye, the perirenal tissue beneath the capsule was slightly or not at all colored despite the fact that its lymph trunks were seen to be deeply colored by the injected dye.

D. *The rate of renal lymph flow.* The total renal lymph flow rate cannot, of course, be obtained from the observed rates of flows of single lymph vessels (table 1), but a maximal approximation, valid for our conditions of observation, may be made. For in no dissection of perirenal tissue following the injection of dye, were there more than ten visible lymph trunks leaving the kidney. Thus a maximal rate of lymph flow may be calculated by multiplying the maximal total number of lymph trunks (ten) by the highest recorded lymph flow (0.0550 gram/min., table 1). This calculation would give the figure 0.5 gram per minute as the maximal total flow for a single kidney. Admittedly, our conditions are so unphysiological that we cannot attach any general significance to this figure, but it is of interest to observe that this rate of 0.5 gram per minute is only about 2 per cent of the total rate of fluid reabsorbtion as calculated from estimates of the glomerular filtrate rate and the rate of urine excretion.

E. *The protein content of renal lymph.* Twelve samples of renal lymph (nine hilar, three external capsular) were collected from eleven kidneys by direct cannulation of a lymphatic vessel leaving the kidney. The average protein content of the twelve samples equalled 1.84 grams per cent, although the individual concentrations of protein varied from 0.44 to 4.21 grams per cent (table 1) and appeared to vary inversely with the rate of flow. For example, in experiment 3a and 3b, the same kidney was cannulated twice. The first lymph flow occurred at a rate of 0.01 mgm. per minute and the lymph had a protein content of 3.0 grams per cent, whereas the second collection from a different vessel gave a lymph flow of 0.035 mgm. per minute with the protein content of 1.41 grams per cent. No striking difference was observed between the protein concentration of external capsular and hilar lymph. It is worthy of note that whenever the rate of flow is not smaller than usual, the protein concentration in renal lymph is lower than the concentration found in lymph from the thoracic duct, liver, lung and heart (6).

F. *The urea content of renal lymph.* Urea determinations were performed upon eleven lymph samples obtained from ten different kidneys. In table 1 it is shown that the urea concentration in the lymph is always higher than the urea concentration in either the arterial or the venous plasma. Special experiments on composite lymph samples showed that the excess "urea" was not ammonia and that it did not arise because heparin was used to prevent lymph clotting, whereas potassium oxalate was employed as an anti-coagulant for the blood. The second last column in table 1 indicates how considerable and yet how variable is the increase in lymph urea concentration relative to that observed in the plasma from the renal vein. The last column gives the reduction in the urea concentration of the venous plasma relative to the concentration in the arterial. This may be taken as a rough index of the urea excretory activity of the kidney, for it has been shown that when the formation of urine ceases this relation is reversed and the vein comes to contain a greater concentration than the artery (9). There is no instance of such a reversal in these observations, though there are three cases in which no difference between vein and artery could be measured. However, there is no constant relation between the increase in lymph urea concentration and the decrease in venous, relative to arterial urea concentrations,

and though the differences in the times of collection of lymph and blood make it impossible for us to attach much significance to this negative fact, the increase in lymph urea is so pronounced as to render it very improbable that variation in renal activity can be the only or the main factor involved. It seems to us that the attempt to explain this interesting fact should be deferred until further experimental evidence has been obtained.

SUMMARY

1. Lymph was collected by cannulation of both capsular and hilar lymphatic trunks. Experiments with dye injections into the renal artery, the cortex, and the medulla indicated that the flow was towards the capsular lymphatics from the cortex and toward the hilar lymphatics from the medulla.

2. The concentration of protein in the renal lymph varied from 0.4 to over 4.0 grams per 100 cc. The slower the lymph flow the greater in general was the protein concentration.

3. The concentration of urea in the renal lymph was always greater, often considerably greater, than the concentration of urea found in the plasma of the renal artery or vein.

The authors wish to express their thanks to Jane Monteith and Eleanor Kruger for technical assistance.

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THE LOCUS AND THE NATURE OF THE A-V PAUSE IN THE SPREAD OF CARDIAC ACTIVATION

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Introduction. One of the inadequately explained phenomena manifested by the vertebrate heart is the junctional delay in the spread of activation. The pause at the atrio-ventricular junction of the mammalian heart has received particular attention in its relation to clinical disturbances of conduction. For the dog heart Hering (1910) concluded that much of the atrio-ventricular delay occurs during the passage of the wave of activation through the node of Tawara. The nature of the process responsible for this delay has, however, remained obscure. Attempts to explain the A-V delay by application of known facts concerning cardiac excitation and conduction have made use of two general types of theory. One involves a concept of latency of ventricular response to the atrial impulse as a stimulus. The other conceives of a delay due to slow transmission through junctional tissue. The possibility that a considerable part of the A-V delay might be attributable to a latency occurring at some point of transmission from one type of tissue to another was suggested by Erlanger (1912). Earlier measurements such as those of Trendelenburg (1911) showed a significant but brief delay between the application of an induction shock and the earliest recorded response but neither these nor later experiments have demonstrated a true latency of response to brief shocks which is long enough to account for the observed junctional delays. Consequently, although arguments have been presented in support of one or another form of "latency" theory they have not met with general acceptance.

In contrast to the latency type of theory are the "transmission" theories which, in logic, have retained essentially the interpretation developed by Gaskell (1900) who postulated that a slower conducting power of the tissues between auricles and ventricles resulted in the observed pause between auricular and ventricular contractions. Ashman (1930) discussed the evidence for the two types of theory and concluded that acceptance of the "latency" theory was unnecessary either for the turtle heart or for the human heart.

It is the purpose of this paper to present material which we believe indicates the possibility of applying a type of "latency" theory, or better, an "excitation time" theory of atrio-ventricular delay to the explanation of this phenomenon. The experimental material deals with electrical excitation of the ventricle and with conduction between the atria and the ventricle, or the reverse, in the heart of the turtle. For most of the experiments we have used the slider terrapin (*P. elegans*). In the turtle the atrio-ventricular connection has been described as a simple muscular junction (Laurens, 1915), the atrial fibers coming into contact with the ventricular fibers. At a room temperature near 25°C. the

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normal A-V delay is about 0.4 second. As a basis for a theoretical interpretation we shall make use of the generally accepted assumption that, for the normally conducted impulse, activation of ventricular tissue is effected by the action potential of the atrium.

Response Times to Stimulation by Electric Currents. a. Procedure. If an "excitation time" theory is to account in any considerable part for the normal range of observed variations in A-V delay it must be shown that the ventricular tissue is capable of responding to applied electric currents after correspondingly long periods of current flow. That is, the minimal demonstrable range of excitation times to applied currents should amount to several tenths of a second. Such excitation times would be impossible if the ventricle developed severe accommodation to applied electric currents or if the ventricle showed only the vanishingly small latencies to currents from auricular tissue indicated by earlier work in which induction shocks were employed as the stimulus to the ventricle. Gilson and Peugnet (1932) reported experiments on turtle ventricular strips which indicated that these did show a considerable degree of accommodation to constant currents. In those experiments the strips were routinely prepared and arranged on electrodes in a moist chamber and preliminary adjustments of switches and other devices were made. This required the passage of fifteen minutes or more and during that time a sufficiently stable condition of excitability was reached so that systematic readings could then be begun. Despite the injury involved in cutting the strips, the absence of perfusion and the repeated elicitation of responses, occasional preparations showed little if any accommodation.

Since that time we have repeatedly observed that the turtle ventricle perfused with suitable oxygenated, buffered saline solution can be maintained for many hours with little sign of deterioration of excitability and that such a preparation, when thoroughly recovered from a previous response, may display but slight accommodation to applied electric currents. In most cases the preparations used in this investigation of excitation times have been maintained by a Straub type cannula, the saline mixture in the cannula being buffered with sodium bicarbonate and bubbled with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. In arranging a preparation, the entire heart was excised, the cannula tip was inserted through the aorta into the ventricle and tied. A ligature was then drawn around the atrial and funnel tissue and tied tightly to prevent conduction from atria to ventricle. With turtles from the laboratory stock, this usually resulted in a quiescent preparation. The material discussed in this section refers exclusively to ventricular preparations in which there were no spontaneous rhythms, or to preparations in which beats appeared at such infrequent times that stimuli could be applied at 30 second intervals with only occasional complications due to spontaneous contraction. The excitability of such a preparation is probably below that of a normal intact and active animal. Stimulating currents were applied through calomel electrodes which showed no significant polarization with test currents (ca. 800 microamperes) much higher than those used for near threshold stimulation (usually 50 to 200 microamperes). Contact with the heart was made by means of a saline soaked bit of yarn which

was placed against the moist heart but was not otherwise attached to it. Amplifier leads were placed intimately about the wick of the cathodal stimulating electrode. In later experiments the cathodal stimulating electrode served as one of the grid lead electrodes constituting one amplifier lead which thus recorded from the point of first response in the heart. The positions of the other two leads were adjusted to give a conveniently small stimulus artefact on the electrographic record. It was sometimes possible to lead directly to the grids of a three-stage differential amplifier coupled with 2 mfd. condensers and 1 megohm grid leak. For the records used for reproduction below, input to the grids of the first amplifier stage was through 0.01 mfd. condensers with half megohm grid leaks.

b. *Constant currents.* Experiments were first carried out using constant currents. Excitation times of 0.4 to 0.7 second were repeatedly recorded. In a few instances significantly longer latencies were recorded. However, to obtain such long time values it was necessary to use current strengths so close to threshold as to make reproducibility of response extremely uncertain. Since a normally beating and conducting heart seems to possess a reasonable margin of safety, and since the cardiac action potentials do not have the form of constant currents, no extensive effort was made to pursue this line of attack.

c. *S-shaped currents.* S-shaped currents were obtained by discharging a condenser connected between grid and cathode of a type 89 tube used as a triode. By use of a variable resistance as grid leak the rate of drainage could be controlled at will. The resulting plate current plotted a curve which rose from cut-off, passed through an early inflection and, for the upper 80 per cent of its course, closely approximated a simple exponential. With such currents, response times of 20 seconds or more have repeatedly appeared and the results are quite reproducible. There is striking similarity of appearance between many of our records and those recently published by Skoglund (1942) for nerve. Obviously such current forms do not reproduce those of the cardiac action potentials. The experiments have, however, demonstrated clearly that the excitation time for response to electrical stimulating currents may be of almost indefinite duration provided that one uses currents of proper form. Considered from a somewhat different aspect, it is seen that the limiting slope for successful excitation of heart muscle may be very slight.

d. *Double condenser shocks.* To achieve a stimulus form more closely resembling that which might be expected of an action potential, somewhat distorted double condenser shocks were obtained by connecting a bridge stimulator (Monnier, 1934) between grid and cathode of a type 89 tube, used as a triode. For shock forms having a rising time measured in seconds or tenths of seconds and with current strengths adjusted to be close to threshold value, the ventricular response occurred at the crest of the wave or before any considerable fall-off had occurred. With shock strengths, increasingly above threshold, the response occurred at earlier times, during the rising phase of the stimulus wave (fig. 1). These results were confirmed by approximate graphic construction using the subtangent method of Rushton (1937).

Locus and relative duration of the junctional delays. a. *Use of electrograms*

obtained by differential leads. To determine the precise locus of the delay which occurs in the transmission of activation across the atrio-ventricular junction, arrangements were made to obtain records from points as close as possible to the actual junction. Using excised turtle hearts the ventral surfaces of atria and ventricle were removed by cutting with fine pointed scissors. The preparation could thus be opened out on wax so as to expose the inner dorsal surface of atria and ventricle. Close to the A-V junction the connection was cut to leave a conducting bridge which usually consisted of the right portion of the dorsal wall and which was from two to four millimeters in width. Two pairs of needle electrodes, each with a needle separation of about one millimeter, were placed near the junction and were so supported that one pair or the other could be moved for exploration. Although attempts were made to avoid injury by pressure many of the records show indications of some injury effects under one

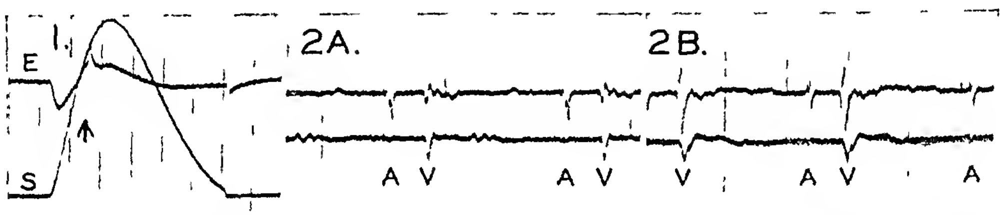


Fig. 1. Excitation time of 1.2 sec. to somewhat distorted double condenser stimulus of greater than threshold intensity. Moment of response indicated by arrow. Perfused turtle ventricle. Time intervals of 1 sec.

Fig. 2. Records obtained by pairs of needle electrodes, each pair having a needle separation of approximately 1 mm. Opened heart with conducting bridge 3.5 mm. wide. Wet preparation with consequent large "extrinsic" lead effects. Time intervals of 1 sec.

A. Electrode pairs separated by 6 mm. Leads for upper record were placed on the atrial side, for the lower record on the ventricular side of the A-V junction. Apparent pause is 0.63 sec.

B. Upper electrode pair was moved downward to within 1 mm. of lower pair, being on the ventricular side of the A-V junction. Apparent A-V pause of 0.6 sec. Records with intermediate electrode positions showed the same A-V pause.

of the needles. Leads from each pair of electrodes were connected to the grids of a differential amplifier which activated a recording galvanometer. A common ground lead was placed, somewhat removed from the heart, on the moist surface of the wax. It was thus possible to place electrodes to touch the heart at desired points and at distances as little as one millimeter from the actual junction. In no experiment in which the preparation showed a normal A-V delay was there found any intermediate junctional region which indicated a significant slowing of conduction. The preparations showed rapid conduction through atrium or through ventricle but an interval essentially that of the full A-V pause occurred between activation of those parts of atrium and ventricle adjacent to the junction (fig. 2).

b. *Effects of stimuli applied above and below the junction.* That normal junctional delay occurs at a sharply localized boundary was indicated even more strikingly by the results of stimulation experiments. When electrodes were

applied to various points on the outside of the intact, perfused heart, close to the atrio-ventricular boundary, stimuli might elicit premature responses of atria and ventricle simultaneously, of atria followed after a junctional pause by ventricle or of ventricle followed after a junctional pause by a response of the atria. At certain times in the heart cycle the conducted responses of ventricle or atrium would not appear but in no case did there appear any junctional pause of intermediate duration as might have been expected under the "transmission" hypothesis which assumes the existence of a tissue having a "slowed conducting

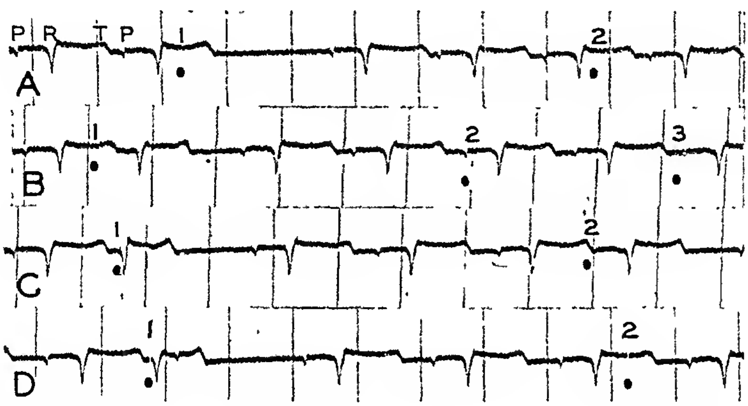


Fig. 3. Effects of position of stimulating electrodes on the response times of the different chambers of the heart. Excised heart, opened ventrally and with A-V connection reduced to 2 mm. bridge. Silver wire stimulating electrodes with 3.5 mm. separation, placed axially and close to the A-V junction. Time of application of stimuli indicated by dots. Heart beating spontaneously and with apparently normal conduction. Time intervals of 1 sec.

A and B. Initial ventricular complex indicated by R, final ventricular complex by T and initial atrial complex by P. Cathodal stimulating electrode toward atrium. Properly timed stimuli caused response of the atrium with (B-1) or without (A-1) conduction to and response of the ventricle. There were no direct responses of the ventricle to the stimulation.

C. Electrode position same as for A and B but polarity was reversed, the cathodal stimulating electrode being toward the ventricle. There were simultaneous responses of both atrium and ventricle (C-1) or of atrium alone (during refractoriness of the ventricle) followed by conduction to and response of the ventricle (C-2).

D. Electrodes moved 1.5 to 2 mm. toward ventricle. Polarity unchanged from that used for C. Atrium was not affected by stimulus. Ventricle responded if not refractory.

The apparent A-V delay is about 0.5 sec. in all cases, no significantly intermediate times having been found in any records.

power". In an attempt to place electrodes more precisely, we again made use of the excised heart pinned out on wax, opened ventrally and with the A-V junction cut down to a narrow conducting bridge. Shocks of brief duration were applied through silver wire electrodes. Results from such an experiment are indicated by the records of figure 3. For record strips A and B, stimulating electrodes having tips separated by 3.5 mm. were placed axially along a 2 mm. conducting bridge and close to the A-V junction. The cathodal electrode was toward the atrium. Atrial premature responses were elicited (fig. 3, responses A-1, B-1) and if the stimuli were properly timed (B-1) there was conduction

across the junction with consequent ventricular response. Direct stimulation of the ventricle did not occur with this electrode position. For strip C, the positions of the stimulating electrodes were unchanged but their polarity was reversed, the cathodal wire being that closer to the ventricle. Stimulation now caused simultaneous responses of atrium and ventricle (C-1), of atrium followed by conduction to and response of the ventricle (C-2) or of ventricle alone according to the excitabilities of the respective chambers at the moment of stimulation. Without change of electrode polarity (cathode still toward ventricle) the electrodes were moved about 2 mm. toward the ventricle and stimulation produced responses only of the ventricle (D-1) never of the atrium. The electrodes were repeatedly passed back and forth across the junction and the results were invariable. If only one chamber (atrium or ventricle) responded to the stimulus directly, there might be conduction to the other chamber but if this occurred it required the full expected time of normal junctional delay. There were never any records showing intermediate shorter times of delay such as would be expected under the "transmission" type of theory. The fact that one could stimulate atrium during the absolutely refractory phase of the ventricle (B-1) and still have conduction across the junction to the ventricle with ventricular activation offers further food for thought in this connection.

Individually and collectively the records of differential lead electrograms and the stimulation experiments offer strong evidence that the junctional delay in transmission between atrium and ventricle occurs at a sharply delimited locus. It is presumed that this locus is identical with that of the anatomical A-V junction. This conclusion is further strengthened by visual observation of active preparations studied under the dissecting miscroscope. Particularly striking was the sort of observation made when there was more or less independence of atrial and ventricular rhythms. In one case, for example, there occurred a brief period of ventricular fibrillation during which there was complete or nearly complete A-V dissociation. Contraction of atrial fibers could be seen on the one side and of ventricular fibers on the other side of the junction. The intermediate region of extinction involved at most a small fraction of a millimeter.

Comparison of Junctional Delays Occurring with Normally Directed and with Retrograde Conduction. Experiments were performed to investigate the relationships existing between junctional delays for normally conducted and for retrograde impulses. Using a perfused, excised heart, lead electrodes were placed on atrium and ventricle and a pair of stimulating electrodes were placed on the ventricle. The heart was allowed to beat spontaneously with normally directed, A-V conduction and then, using a brief condenser discharge as stimulus, the ventricle was stimulated at a rate slightly faster than that of the normal rhythm. With proper phase relations between stimulus and the beat of the normal pacemaker, ventricular beats were conducted in a retrograde direction and caused responses of the atria. One record obtained by this method showed the A-V interval to be 0.44 sec. The V-A interval was 0.73 sec. Such times are typical although in some preparations the A-V and V-A delays may approach even one another more closely.

The record of another example of retrograde conduction is shown in figure 4. An atropinized heart was pinned out on wax and opened ventrally, the A-V junction was reduced to a 2 mm. bridge and stimulating electrodes were so placed that the cathode was about 2 mm. on the ventricular side of the A-V junction. The heart continued to beat spontaneously and the A-V delay was 0.48. When the ventricle was stimulated so as to cause retrograde conduction the V-A delay was about 0.9 sec. Some variation due to fatigue, etc., is indicated. Coming after the initial atrial deflection (P-wave) which follows the ventricular response numbered 5 there may be seen a slight deflection equivalent to an atrial T-wave. The normal A-V pause is such that ventricular activation first occurs during the atrial T-wave. Furthermore it is to be seen that where retrograde conduction occurs (fig. 4, following ventricular responses 2, 4 and 5) there is beginning activation of the atrium during the ventricular T-wave. With due allowance for conduction times the time of the crest of the T-wave corresponds to the moment of most rapid fall of a monophasic action potential and it

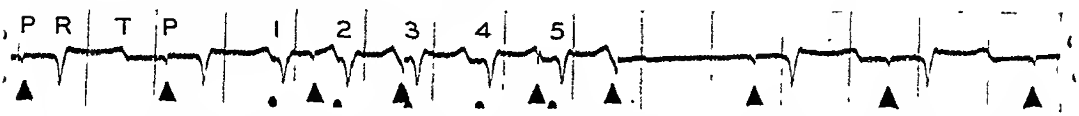


Fig. 4. Retrograde conduction recorded from same preparation as that used for records of figure 3. Cathodal stimulating electrode on ventricular side of the junction. Times of application of stimuli indicated by dots. Responses of atria indicated by triangles.

Shock 1 caused premature response of ventricle, no retrograde conduction.

Shock 2 caused response of ventricle with retrograde conduction to and response of the atrium.

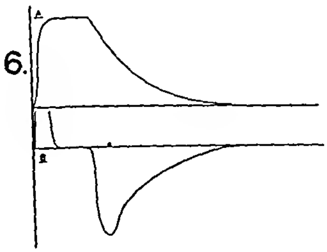
Shock 3 stimulated the ventricle but produced no apparent effect on the atrium.

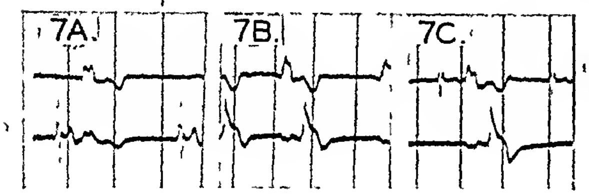
Shocks 4 and 5 stimulated the ventricle and there was retrograde conduction to the atria in each case. The atrial response following response 5 of the ventricle shows a barely perceptible atrial T-wave.

will be seen in the theoretical section below that this is approximately the time at which transjunctional stimulation would be expected. In a large number of records taken at room temperature and showing clear atrial or ventricular T-waves this relationship has held without exception.

Figure 5 represents in schematized form the condition which may exist at a small region of the atrio-ventricular junction. It is of fundamental importance to the interpretation of the diagram that the ventricular element be considered as completely external and extracellular to the atrial element. Cellular continuity and relatively rapid, continuous conduction may exist throughout atria or throughout ventricle but is believed to be effectively interrupted at the atrio-ventricular junction. Elements of atrium (A-C) and of ventricle (C-D) are diagrammed as lying on either side of a simple atrio-ventricular junction (C) and to be lying in a plane conducting medium. The atrium may be considered to be in one of two arbitrary conditions: 1, with the advancing wave of atrial *excitation* at a point half way along the diagrammed atrial element, or 2, with the advancing front of *recovery* half way along the atrial element. It is recognized that both the times selected and the sharpness of demarcation at the boundary

B are arbitrary and they have been chosen only for convenience in diagramming and description. In more general terms, the first condition may be regarded as occurring at any moment during the rise and spread of electrical activity





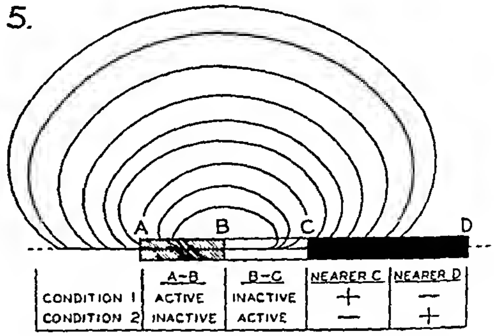


Fig. 5. Diagram indicating the direction of external electrotonic current lines (drawn only for the upper half of a schematic plane conducting field) resulting from the atrial action potential during times for:

Condition 1, when the atrial element (A-C) is relatively active at the left (A-B) and still relatively inactive at the right (B-C); and

Condition 2, when the atrial element is relatively recovered at the left (A-B) and is still relatively active at the right (B-C).

The adjacent ventricular element (C-D) is considered to be completely external to the cell structure comprising the atrial element and to be in a resting state for both conditions of the diagram.

It will be seen that during the arrival and development of the atrial action potential at such a junction of abutment (at C), there is local relative positivity of that part of the surface of the ventricle near C (condition 1). During the period of electrical recovery which roughly coincides with the fall of the monophasic action potential of the atrium (condition 2) there is local relative negativity of that part of the surface of the ventricular element near C and ventricular excitation would be expected to occur during this period.

Fig. 6. Curve A. Schematized diagram of cardiac (atrial) monophasic action potential.

Curve B. Plot of differential curve of A. If the second (downward) phase of B represented the plot of a stimulating current of just threshold value, response of the stimulated (ventricular) element would be expected at or very shortly after the moment indicated by the black dot.

Fig. 7. Records from depressed preparation which displayed long apparent A-V pause to show intermediate response of tissue near A-V junction occurring between responses of atrium and of ventricle. Paired silver wire lead electrodes were placed to touch lightly against the preparation.

A. Leads from right atrium (lower record) and from intermediate region at A-V junction (upper record).

B. Leads from junctional region (upper record) and from points near the middle of the anterior ventricular surface (lower record).

C. Leads from atrium (upper record) and from ventricle (lower record).

through the atrium. The second condition may in its general sense represent the situation at any time during the recovery of the atrium toward the electrical resting state. The atrial element is considered as being first activated at the

left end of the diagram. Schematic lines indicating flow of electrical current have been drawn only for the upper half of the plane figure.

For condition 1, representing a time during the activation of the atrium, the region at the left of B is negative to the region at the right of B. The orientation of potential differences along the ventricular surface under this condition is not such as to result in ventricular stimulation at C during the arrival or rise of the action potential at the A-V junction. The condition which might apply at an instant when complete activation of the entire atrium has been achieved and the entire atrial surface has reached an equi-potential state, if it exists at all, is very transient. During this time no current will flow from one part of the atrium to another. Consequently no electrotonic currents will be set up along the ventricle. No potential differences will be established along the surface of the resting ventricle and no stimulating effect will be manifested during this period. A third possibility is indicated by the second condition of figure 5. The electrotonic currents set up across the recovering-active boundary of the atrium will establish potential differences along the ventricular element which will be so oriented that the ventricular region nearer C will be negative to the region nearer D. Consequently, if these potential differences were developed with proper time and intensity functions, transjunctional stimulation of the ventricle would be expected to occur.

It is evident that the potential changes which are established along the ventricular surface are affected by the form of the atrial action potential. Thus, disregarding conduction, an approximation to the curve of potential which results in stimulation of the ventricle can be had by plotting the differential curve of the monophasic atrial action potential, i.e., the curve of potential differences, at successive times, between the terminal region of the atrial element and a second region on the atrium just to the left of it, or, and more properly, a curve of the slopes, at successive times, of the monophasic action potential. Such a pair of curves have been diagrammed as figure 6. Curve A represents a highly schematized monophasic action potential. Curve B plots the differential curve of A. The vertical ruled line indicates zero time for the two curves. For a monophasic curve of the form plotted, and with an intensity of atrial action potential such as to be barely above threshold for ventricular stimulation, ventricular response would be expected to occur at, or very shortly after, the time indicated by the black dot (see section dealing with double condenser stimuli). The local atrio-ventricular delay would therefore be approximately the time from the zero time line to the dot. An atrial action potential of greater than threshold value would stimulate at a time earlier than that indicated by the dot but not at a time earlier than the beginning of the downward phase of curve B. An increase of steepness of the recovery phase of the monophasic curve would, other things remaining constant, be expected to give a shortened A-V delay. A flattening of this curve would correspondingly be expected to increase the A-V delay. Lengthening or shortening of the duration of the monophasic action potential would likewise, other things remaining constant, be expected to lengthen or shorten the A-V pause. Such variables can be predicted from

experiments with double condenser stimuli and the range of excitation times found in those experiments are more than adequte to account for the normal time range of observed junctional delays.

A-V Conduction with Long Delay. The material presented above does not take into account the long A-V delays which are often seen in the laboratory. At temperatures of about 25°C. such delays are frequently about 0.8 sec. in duration but may be longer. In attempts to obtain preparations showing such long times between responses of atria and ventricle several procedures were tried, one of the most satisfactory being as follows. The atropinized heart was excised and removed to a shallow dish containing a thin layer of saline. The ventral opening of atria and ventricle was carried out as described above and a sagittal or parasagittal cut was then made through the ventricle and into the atria. The ventricle was thus completely divided into two parts both of which were activated by impulses which arrived over more or less normal paths from the sinus pacemaker. If necessary, further embarrassment of A-V conduction was accomplished by reducing the width of the conducting bridges between atria and ventricle. After the sagittal cut had been made one part of the ventricle, usually the left, generally remained in standstill for some minutes and then resumed a conducted response, frequently with partial block and frequently with a significant lengthening of the A-V pause. It became evident that the long A-V times whieh were recorded in these preparations either were or closely approached being simple multiples of the shortest A-V time which was actually observed or which might have been expected as normal on a basis of previous experience. That is, using a hypothetical example, if the A-V time of the freshly exposed heart was 0.4 sec., the A-V pause for the right side of such a maltreated preparation might be 0.8 sec. and that for the left side might be 1.2 sec. If premature stimuli were applied to the atria, conduction to the right ventricular portion might require 1.2 or 1.6 sec., and conduction to the left ventricular portion might require 1.6 or 2.0 sec.

Such results presented but two simple alternatives. Either the conclusions drawn from the preceding sections were untenable or additional finite increments of delay had entered the system. The latter proved to be the case. An opened, cut heart from which records had been obtained was studied under the dissecting microscope. Between atria and left ventricle two other serially activated, discrete responses could be seen to occur within a region extending 1.5 to 2 mm. from the A-V junction. Partial blocks existed between each of these four increments so that at one counting the rhythms were 2:1, 3:1, and 2:1, respectively. Thus, for the period considered, there were 12 beats of the atrium to 1 beat of the ventricle. The electrical records which had been made a few minutes previously showed an A-V delay 4 times the normal, probably indicating either that one responding increment was not seen or that there had been a change in the number of functional mechanisms responding. For the right part of the same preparation, electrical records had indicated a delay 3 times the normal. One intermediate responding element was clearly observed.

Electrical records were made from such preparations, and, if electrodes were

carefully placed, the recorded electrograms showed responses occurring at times predictable as multiples of the normal junctional delay for the freshly exposed heart. In most cases movement of the electrode by a millimeter was sufficient to bring in or completely lose the recorded response of the small intermediate increments. Occasionally a considerably larger part of the endocardial surface of the ventricle was observed to show movement, an observation first made in the case of a preparation which displayed partial block between this region and the rest of the ventricular mass. Figure 7 presents records from a preparation showing such an extensive intermediate region. A response was recorded by electrodes placed on the ventricular endocardium or the ventricular ring at a time roughly midway between the times of responses of atrium and of ventricle. The laboratory temperature was 32°C. at the time and other records taken from the preparation indicate that the delay times apparent in these records (about 0.6 sec.) are twice the duration of the unit delay time for the preparation. In most cases the time functions recorded indicated that the intermediate responses have properties of atrial rather than of ventricular tissue. A few ambiguous cases may involve delays longer than this and may be concerned with delays of truly junctional type appearing between increments of ventricular tissue. Study of microscopic sections cut from one preparation which had been carefully observed in action indicated that the most critical region for the appearance of delays intermediate between atrium and ventricle is that in which atrial fibers are entering the ventricle and establishing contact with the muscular ring at the ventricular base.

Discussion. The repeated observation that long A-V delays are associated with the introduction of one or more visibly and electrically responding increments interpolated between atrium and ventricle makes it possible to explain such long delays under the hypothesis presented above. The observation also offers a possible explanation for the very long A-V times which have been reported in the clinical literature (see Barker and Bridgman, 1917; Herrmann and Ashman, 1926) and which have been difficult to explain under the classical theories of junctional delay.

A clinically recognized phenomenon representing the opposite extreme from the very long A-V times is that of the short P-R interval found with the syndrome of Wolff, Parkinson and White (1930). The short P-R interval has been interpreted by Wolferth and Wood (1933) as possibly due to the presence of an aberrant or accessory atrio-ventricular conducting bundle. Recently Butterworth and Poindexter (1942) have reported experiments which they considered as confirmatory of this interpretation. The latter workers used the amplified action potential led from the dog or cat atrium as a stimulus to activate the ventricle. The resulting electrocardiograms showed great similarity to those recorded clinically from individuals showing the Wolff-Parkinson-White syndrome. In the light of the material which we have reported above, it appears that the highly significant aspect of the experimental results of Butterworth and Poindexter lies not in the introduction of an aberrant pathway to cause stimulation of the ventricle but rather in the fact that the accessory path was

such that stimulation of the ventricle occurred without the usual junctional delay. It seems probable that if a few conducting fibers in the heart of occasional humans were to develop anatomical relationships or excitability characteristics such as to make possible an effective short-circuiting of the usual truly junctional connections in the A-V node, there would be precisely the shortening of P-R interval which is actually seen. A reverse phenomenon would result in abnormally long A-V delays. The abnormality then would be one concerned with delicate anatomical or excitability relationships between connecting cardiac fibers rather than one involving the presence of actual accessory pathways for conduction. The belief that such may be the case is heightened by the variety of disturbances in rhythm which may be developed experimentally in the heart by use of a technic similar to that described by Butterworth and Poindexter. These disturbances closely simulate conditions which have been recorded clinically.

The experimental results which we have obtained indicate that in the turtle conduction from atrium to ventricle, as observed normally, depends upon the use of a path involving a single junctional delay. That this path may not always be the same anatomically is indicated by the conflicting results found in the earlier literature on the subject. In our experience there have been instances of profound and even permanent A-V block resulting from small incisions into the right lateral part of the A-V connection. On the other hand a small injury into the left region has on two occasions proved equally serious, the right side which remained intact conducting only infrequently and with long A-V delay. Changes in heart rate will cause minor changes at the normal, single junction which may, of course, involve the activity of extremely few fibers. In the depressed, prematurely activated preparation spread of excitation by this path involving only the single junctional delay may fail. Paths involving the use of two or more junctions may then come into play and the measured A-V interval will show a corresponding multiplication of the apparent delay. It seems not impossible that transjunctional conduction in other types of heart or in other tissue complexes may involve functional mechanisms similar to those which we have studied.

Summary. 1. We have reported above upon experiments which were performed in an attempt to determine the locus of the atrio-ventricular delay in the heart of the turtle and to determine the cause or nature of this delay in transjunctional conduction.

2. Electrical currents of several forms have been used in stimulation of excised perfused ventricles. Slowly rising S-shaped currents or currents approaching double condenser waves in form were found to give excitation times which could be many seconds in duration.

3. Electrical records of normally conducted impulses, results of experiments with electrical stimulation eliciting premature responses and visual observation all lead to the conclusion that the normal A-V delay occurs *at* the locus of the atrio-ventricular junction.

4. During normal A-V conduction, the beginning of ventricular electrical activity was found to occur during the atrial T-wave.

5. It is suggested that an "excitation time" theory may account for the normal delay in impulse propagation across the atrio-ventricular junction of the turtle heart.

6. Because of the direction of the lines of electrotonic current flow along the surface of a ventricular element which is considered as being completely external and extracellular to an abutting atrial element, ventricular excitation would normally not be expected to occur during the arrival or rise of the atrial action potential at the junction. With such a fiber arrangement ventricular response would occur at or before a moment roughly that at which there is the most rapid recovery of the responsible atrial element, immediately adjacent to the junction.

7. Preparations showing long A-V delays which approach multiples of the normal value have been observed to show responses of small tissue regions which were localized near the A-V junction and which showed onset of activity at times intermediate between those of atrial and of ventricular responses.

8. It is suggested that the short P-R interval of the Wolff-Parkinson-White syndrome may represent atrio-ventricular conduction without the usual, normal junctional pause in the spread of excitation.

CONCLUSIONS

It is concluded that an "excitation time" theory may be used to explain the delay normally occurring in the transmission of the cardiac impulse across the atrio-ventricular junction in the turtle heart. Ventricular excitation normally does not occur during or because of the arrival and rise of the atrial action potential but during the period of electrical recovery of the atrium. Long atrio-ventricular delays may involve serial activation of several elements thus multiplying the single junctional pause found in the normal heart.

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THE APPARENT VOLUME OF DISTRIBUTION OF SULFOCYANATE AND OF SULFANILAMIDE IN THE DOG[1]

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A number of substances, including sodium, chloride, bromide, sulfate, sucrose and sulfocyanate (1, 2, 3, 4) appear in the main to be distributed, following injection, through only the extracellular portion of the body fluids. Of these substances sulfocyanate is most frequently used to measure extracellular fluid volume because of the apparent ease of its determination and its slow excretion.

The distribution of sulfocyanate, however, has usually been followed for only a few hours after injection and only after single injections. The degree of variations in the apparent volume of distribution over longer periods and after multiple injections has not been clearly delineated. Study of such variations in dogs was undertaken because repeated measurements of extracellular fluid volume were desired in experiments extending over many days.

Crandall and Anderson (4) determined the sulfocyanate distribution volume in dogs 2 to 4 hours after injection. Lavietes, Bourdillon and Klinghoffer (3) obtained a constant volume in human subjects as long as 24 hours after injection. No study has been found of the volume of distribution of sulfocyanate in the dog over a similar period of time.

In six preliminary determinations of the volume of distribution in four dogs at intervals of 16 to 21 hours after injection, values were obtained ranging from 29 to 40 per cent of the body weight with an average of 36.8 per cent. In four determinations at 4 hours the average volume was 32.7 per cent of the body weight. This latter value is identical with that obtained by Crandall and Anderson as the average in 33 dogs, and is somewhat closer to the values calculated from the total water and electrolyte content of whole dogs (1). These findings suggested that the apparent volume of distribution of sulfocyanate in the dog increased with time.

Painter (5) has reported that the volume of distribution of sulfanilamide in the dog may be used as a measure of the total body water. Heinemann (6), however, found that sulfanilamide is not distributed evenly between serum and cells of human blood. If such is the case in dog blood in vivo, the distribution of sulfanilamide, calculated as Painter does, from its concentration in whole blood, cannot be used as a valid measure of total body water. It seemed worthwhile, therefore, to reinvestigate this problem, taking care to determine separately the concentration of sulfanilamide in cell water and in serum water.

METHODS. Sulfanilamide was determined in serum and in urine by the

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[2] National Research Council Fellow in the Medical Sciences.

method of Bratton and Marshall (7). For the determination of sulfocyanate the method of Crandall and Anderson (4) was modified as follows:

The concentration of sulfocyanate was determined from the depth of color developed with ferric nitrate reagent. In the preliminary experiments referred to above, the reagent was added to a trichloroacetic acid filtrate of serum and to urine from which the pigment had been removed with animal charcoal, and the depth of color read in a visual colorimeter. The animal charcoal was later found to remove some of the sulfocyanate.

Therefore, to obtain more reliable determinations of sulfocyanate for the present experiment the method was adapted to the Evelyn photoelectric colorimeter.

Reagents. 1. Ten per cent trichloroacetic acid. 2. Ferric nitrate reagent consisting of: 25 grams of $Fe(NO_3)_3 \cdot 9H_2O$, 12.5 cc. of HNO_3, 500 cc. of water.

Serum. A protein-free filtrate of serum was obtained by adding 6 cc. of 10 per cent trichloroacetic acid to 2 cc. of serum. Four cubic centimeters of the filtrate were transferred to a calibrated colorimeter tube, followed by 4 cc. of distilled water and 2 cc. of ferric nitrate reagent, making a total volume of 10 cc. A blank was prepared simultaneously consisting of 5 cc. of distilled water, 3 cc. of 10 per cent trichloroacetic acid, and 2 cc. of ferric nitrate reagent. The readings were made with a filter of 490 μ.

Urine. Five-tenths cubic centimeter of urine, 7.5 cc. of distilled water, and 2 cc. of ferric nitrate reagent were pipetted into a colorimeter tube making a total volume of 10 cc. This solution was a 1:20 dilution of the urine. At this dilution the effect of urine pigment was found to be negligible when the undiluted urine was of a specific gravity of 1.010 or less. The blank was made up of 8 cc. of water and 2 cc. of ferric nitrate reagent. The readings were made with the filter of 490 μ.

A blank substance which produced color with the ferric nitrate reagent, but which was not oxidizable under the same conditions as sulfocyanate, was found in the urine of dogs which had received no sulfocyanate. The concentrations of this substance in the urine varied directly with the specific gravity, and interfered with the recovery of added sulfocyanate when the specific gravity of the urine was higher than 1.005. Because of this fact and because of urinary pigment unknown urines were initially diluted 1:5 or 1:10 to a specific gravity of 1.005 or less. This dilution plus the second 1:20 dilution in the colorimeter tube made a final dilution of 1:100 or 1:200.

In 27 urine specimens from 7 normal dogs it was found that for every 0.001 of specific gravity the average color value of this blank substance was equivalent to that produced by 0.146 mgm. per cent of sulfocyanate with a standard deviation of ± 8.0 per cent. From this relationship a value for the blank was calculated for each unknown urine. The accuracy of recovery of known amounts of sodium sulfocyanate added to 37 urine specimens with a dilution of 1:100 or 1:200 was ± 5.0 per cent.

A small blank of a magnitude equivalent to 0.1 to 0.4 mgm. per cent sulfocyanate was found in the serum of dogs which had received no sulfocyanate.

This blank, however, did not appear to be additive to sulfocyanate added to the serum in vitro. If the blank was excluded, the recovery of the sulfocyanate varied from 105 to 96 per cent. If the blank was included the recovery was 93 to 98 per cent. For the purposes of the experiment this blank was considered not to be additive and the accuracy of the determination was taken to be ±5 per cent. The behavior of the blank remains an enigma.

PROCEDURE AND CALCULATION. Sodium sulfocyanate in 1 per cent solution was injected intravenously into the dog from a calibrated burette. At each time for the determination of the volume of distribution a sample of blood was taken from the jugular vein, the urine was collected from the metabolic cage, the animal was catheterized and the bladder washed several times with isotonic saline solution. The feces of dogs 3 and 4 were collected over the entire period of the experiment and analyzed; a negligible amount of color-producing substance was present. Anesthesia was not required.

The calculation of the volume of distribution of diffusible sulfocyanate presents some difficulties. Lavietes, Bourdillon and Klinghoffer (3) found that the concentration of sulfocyanate was approximately 10 per cent higher in serum than in transudates in patients given sulfocyanate. Rosenbaum and Lavietes (8) showed that in human serum a fraction of sulfocyanate is bound to lipoid and is not present in the ultrafiltrate. To allow for this non-diffusible fraction in serum and for the entrance of sulfocyanate into the red cell, the former authors constructed an equation which was felt to represent more nearly the extracellular fluid volume. This equation was not used for the calculation in our experiment because no direct measurements were available of the blood volume and serum volume.

The apparent volume of distribution of any substance theoretically may be calculated from the following equation:

$$\text{Apparent volume of distribution} = \frac{\text{Amount given} - \text{amount excreted}}{\dfrac{\text{Rise in serum concentration}}{\text{Water content of serum}} \times \text{Donnan factor} \times (1\text{-Fraction bound})}$$

Theoretically, the Donnan factor and (1—fraction bound) are separate entities, the latter depending upon the amount of substance so bound to protein or lipoid that it will not pass through the capillary membrane. As actually measured by ultrafiltration the diffusible fraction is the product of these two factors. For sulfocyanate in dog serum the diffusible fraction is approximately 0.83 x serum water concentration (9). This factor in part cancels the correction for the water content of serum, 1:0.93. For this reason and since no better methods of calculation are available at present, the following simplified equation was used for the calculation in this experiment:

$$\text{Apparent volume of distribution} = \frac{\text{Amount given} - \text{amount excreted}}{\text{Rise in serum concentration}}$$

Sulfanilamide in 0.6 per cent solution was injection and recovered in the same manner as sulfocyanate. As sulfanilamide is excreted much more rapidly than

sulfocyanate, the theoretical volume of distribution at the time of injection was calculated from a concentration value obtained by extrapolation of the con-

TABLE 1

The apparent volume of distribution of sodium sulfocyanate in two dogs during 96 hours after three successive injections

DOG	WEIGHT	TIME: After initial injection	TIME: After immed. injection	NaSCN: Amount given i.v.	NaSCN: Serum conc.	NaSCN: Output: Blood	NaSCN: Output: Urine	NaSCN: Net retention	NaSCN: Volume of distribution	
	kgm.	*days*	*hours*	*mgm.*	*mgm. per cent*	*mgm.*	*mgm.*	*mgm.*	*liters*	*per cent body weight*
1	16.24	0	0	466	0.15					
			4		10.85		11	455	*4.19*	*25.8*
	16.10	1	24		6.80	1	107	347	5.10	31.7
	16.18	2	48		4.07	1	108	238	5.85	36.2
	16.12	3	72		2.62	1	64	173	6.60	40.9
	15.82	4	96		1.75		29	144	8.23	52.0
		10			0.34		51*	93		
	15.66	10	0	466						
			4		11.35		1	465	*4.10*	*26.2*
	15.54	11	24		9.90	1	58	406	4.10	26.4
		12	48			2	95	309		
	15.22	13	72		7.07		33	276	3.91	25.7
	15.66	14	96		5.10	1	44	231	4.53	29.0
		14	0	280						
			4		10.53	1	51	228	*4.19*	*26.8*
2		0	0	493	0.19					
	15.96		4		8.97		19	474	*5.29*	*33.2*
	16.30	1	24		4.60	1	168	305	6.63	40.7
	16.40	2	48		1.29	1	117	187	14.5	88
		3	72				10	177		
	15.76	4	96		0.29		19	158	54.5	
			0	491						
	15.74		4		7.92		70	421	*5.31*	*33.7*
	16.20	5	24		3.06	1	117	303	9.90	61.1
	16.40	6	48		1.26	1	57	245	19.5	
		15	0	493	0.17					
	17.00		4		8.05		7	486	*6.08*	*35.3*
	17.10	16	24		4.95	1	149	336	6.79	39.7
		17	48			1	170	165		
		18	72		0.59		49	116	19.6	

* Sulfocyanate had disappeared from the urine by the 10th day.

centration attained over the first four hours after injection. The apparent volume of distribution was calculated in three ways, from the concentration of

sulfanilamide in the water of serum, in the water of the red cells, and in the water of whole blood respectively. For the last two calculations the following simple equation was used:

$$\text{Apparent volume of distribution} = \frac{\text{Amount retained}}{\text{Concentration in mgm. per liter of water}}$$

However, for the calculation based on the concentration of sulfanilamide in serum water it was necessary to introduce another factor. Heinemann (6) has shown that only 75 per cent of sulfanilamide in human serum is ultrafiltrable

TABLE 2

The apparent volume of distribution of sodium sulfocyanate in two dogs during 96 hours after a single injection

DOG	WEIGHT	TIME AFTER INJECTION		NaSCN						
				Amount given i.v.	Serum conc.	Output		Net Retention	Volume of distribution	
						Blood	Urine			
	kgm.	*days*	*hours*	*mgm.*	*mgm. per cent*	*mgm.*	*mgm.*	*mgm.*	*liters*	*per cent body weight*
3		0	0	260	0.10					
	10.98		4		6.18	1	0	259	4.18	38.1
	10.92	1	24		4.62	1	58	200	4.33	39.7
	10.78	2	48		2.73	1	91	108	3.96	36.8
	10.88	3	72		1.88		25	83	4.42	40.6
	10.84	4	96		1.38		27	56	4.06	37.4
		14					95	−39		
4		0	0	260	0.15					
	11.28		4		7.18	2	0	258	3.59	31.8
	11.38	1	24		4.95	1	45	212	4.28	37.6
	11.76	2	48		2.50	1	93	118	4.72	40.1
	11.46	3	72		1.73		15	103	5.95	52.0
	11.40	4	96		1.36		52	51	3.75	32.9
		14					116	−65		

the rest being bound presumably to protein. Therefore the following equation was used:

$$\text{Apparent volume of distribution} = \frac{\text{Amount retained}}{\text{Concentration in mgm. per liter serum water} \times 0.75}$$

The water content of whole blood was determined directly by the difference between wet and dry weights, that of the red cells was calculated from the water content of whole blood and the cell volume. The water content of serum was calculated from the total protein concentration.

RESULTS. The volume of distribution of sulfocyanate was followed after three successive injections in two dogs over periods of 24 to 96 hours (table 1).

The values obtained at 4 hours after injection were fairly constant in each dog. With the exception of the second injection in dog 1, the volumes at 24 hours were definitely larger than at 4 hours, and increased steadily up to 96 hours. The values obtained after the second and third injection were calculated on the assumption that all of the sulfocyanate excreted was from the immediately preceding injection only. If a portion were derived from previous injections, the volumes obtained, and hence the discrepancies, would be even larger.

TABLE 3

The apparent volume of distribution of sodium sulfocyanate in two dogs at one, two, and four hours after three successive injections

DOG	WEIGHT	DATE	TIME AFTER INJECTION	NaSCN Amount given i.v.	Serum conc.	Output Blood	Output Urine	Net retention	Volume of distribution	
	kgm.		*hours*	*mgm.*	*mgm. per cent*	*mgm.*	*mgm.*	*mgm.*	*liters*	*per cent body weight*
3		May 25	0	289	0.20					
	11.04		1		7.68			289	3.76	34.0
	11.01		2		7.62	2		287	3.77	34.2
	10.94		4		7.38	1	5	281	3.80	34.7
		June 2	0	289	0.17					
	11.50		1		8.25			289	3.50	30.4
	11.70		2		7.43	2		287	3.86	33.0
	11.50		4		7.00	2	17	268	3.83	33.3
		June 29	0	290	0.30					
	10.20		1		8.16	1		289	3.54	34.7
	10.14		2		8.00	2		287	3.59	35.4
	10.10		4		7.79	3	7	277	3.56	35.2
4		May 25	0	289	0.23					
	11.38		1		8.06			289	3.58	31.5
	11.34		2		8.32	2		287	3.45	30.4
	11.28		4		8.16	1	3	283	3.47	30.8
		June 2	0	289	0.12					
	11.52		1		8.85			289	3.26	28.5
	11.42		2		8.44	2		287	3.40	29.8
	11.44		4		8.05	2	17	268	3.33	29.1
		June 29	0	289	0.34					
	10.50		1		9.35	1		288	3.08	29.4
	10.42		2		9.30	3		285	3.07	29.5
	10.36		4		8.75	4	4	277	3.16	30.5

After the second injection in dog 1 the apparent volume of distribution remained at a constant level for 72 hours before increasing. In this dog 79 per

cent of the injected sulfocyanate was recovered by the end of the eighth day after the last injection, but sulfocyanate was still being excreted.

The apparent volume of distribution of sodium sulfocyanate after a single injection was followed for 96 hours in two other dogs (table 2). In dog 3 the values obtained over four days varied only slightly, and in both directions from the 4 hour volume. In dog 4 a large increase in the apparent volume occurred during the first 72 hours. On the fourth day the excretion rate increased and the apparent volume returned to approximately the 4 hour value.

Urine and stools were collected for 14 days after the administration of sulfocyanate. In both dogs more sulfocyanate was recovered than was given, 115

TABLE 4

The distribution of sulfanilamide between cells and serum in dog blood in vivo, and the apparent volume of distribution of sulfanilamide calculated from the respective concentrations in whole blood water, in serum water, and in red cell water

DOG	TIME	WEIGHT	AMOUNT GIVEN	SULFANILAMIDE									
				Concentration			Net retention	Volume of distribution					
				Blood water	Serum water	Red cell water		Blood water		Serum water		Red cell water	
	hour	*kgm.*	*mgm.*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm.*	*liter*	*per cent body weight*	*liter*	*per cent body weight*	*liter*	*per cent body weight*
5	0	5.57	240	7.0*	5.0*	10.3*	240	3.4	62	6.3	113	2.3	42
	1			6.1	4.5	8.8							
	2			5.1	3.9	7.2							
	4	5.52		4.4	3.0	6.8	183	4.2	76	8.0	145	2.7	49
	12	5.72		1.4	0.7	2.6	101	7.2	126	20.2	354	4.0	70
6	0	7.30	600	11.3*	9.1*	15.1*	600	5.3	73	8.8	121	4.0	55
	½			11.0	8.9	14.8							
	1			9.6	7.9	13.1							
	2			8.7	7.1	11.7							
	3			8.1	6.5	11.2							
	4	7.30		7.2	6.1	9.1	509	7.0	96	11.1	152	5.6	77
	12	7.20		4.1	3.2	5.6	338	8.3	115	14.1	196	6.0	83

* Values obtained by extrapolation.

per cent and 125 per cent in dogs 3 and 4 respectively. Only a negligible amount of sulfocyanate was recovered in the stools.

In the same two dogs the apparent volume of distribution of sulfocyanate was measured at 1, 2, and 4 hours after each of three successive injections (table 3). The volume at 1 and 2 hours was calculated by dividing the amount given by the serum concentration. At 4 hours the calculation was modified by subtracting from the amount of sulfocyanate given the amount excreted in the urine (as obtained by catheterization). The values obtained indicated a fairly uniform volume during the first four hours. In the one exception, the second injection of dog 3, through an error the animal was allowed a drink of water.

The results as related to body weight were also in good agreement for all three injections.

Sulfanilamide was found to be unevenly distributed between serum and cells in the blood of two dogs in vivo (table 4). The concentration in cell water was about twice that in serum water. The apparent volume of distribution at the time of injection, when calculated from the concentration in serum water was 113 per cent and 121 per cent of the body weight, and when calculated from the concentration in red cell water was 42 per cent and 55 per cent of the body weight. When calculated from the concentration in the water of whole blood, the values obtained were 62 per cent and 73 per cent of the body weight. The apparent volume of distribution as calculated by all three methods progressively increased in magnitude at the fourth and twelfth hours after injection. Throughout the experiment no acetylated sulfanilamide was found in blood or in urine.

Discussion. The apparent volume of distribution of sulfocyanate appears to be consistently reproducible in any given dog *during the first four hours* after any number of injections. No evidence is presented as to the exact relation of this volume to the true extracellular fluid volume, and hence the former can only be taken as a measure of the approximate, but not the absolute, magnitude of the latter. But *changes* in the distribution volume of sulfocyanate as determined during the first four hours after injection may reasonably be taken to measure *changes* in the true extracellular fluid volume. For this purpose it should be a valid experimental technique.

The evidence presented, however, indicates that *after* the first four hours following injection the volume of distribution of sulfocyanate is no longer an accurate measure of any one fraction of the body fluids. The increase of the apparent volume of distribution over time in these dogs indicates a discrepancy between the apparent volume and the true volume of distribution because there was no valid reason to believe that the latter was increasing. To explain this discrepancy it is necessary to examine the assumptions on which the calculation of the apparent volume of distribution is based.

The calculation of the volume of distribution of an injected substance depends upon the following assumptions, as stated by Winkler and Smith (10): "1) that the substance is neither formed nor destroyed in the body; 2) that it is uniformly distributed throughout some portion of the body water; 3) that the concentration of the water of serum is a fair sample of its concentration throughout this portion; 4) that it is excreted solely by way of the urine" and feces.

The experimental evidence indicates little if any destruction of sulfocyanate in the dog. The first assumption, therefore, appears valid to the extent that sulfocyanate does not appear to be destroyed. If any is formed in the body it must be in amounts too small to affect the course of the experiment.

The accuracy of the modified method and the complete recovery over time of the injected substance would appear to preclude recovery from the urine and feces as an explanation of the discrepancy. The fourth assumption likewise appears valid.

The fault must, therefore, lie with one or both of the two remaining assumptions. Either sulfocyanate is not uniformly distributed throughout some portions of the body water, or its concentration in the water of serum is not a fair sample of its concentration throughout this portion. The latter possibility might explain an error in calculating the *absolute* magnitude of the extracellular fluid, but it could hardly explain an *increasing* apparent volume of distribution. On the other hand a rapid diffusion of sulfocyanate into the extracellular fluid, followed subsequently by a slow entry of sulfocyanate into some tissue phases not in equilibrium with the extracellular fluid would readily explain this rising apparent volume. Sulfocyanate is known to enter erythrocytes and some cells of the gastrointestinal mucosa (3, 4), so such an explanation is not without experimental support. The constancy of volume for 72 hours following the second injection of sulfocyanate in dog 1 is consistent with a "saturation" of such a cellular depot, while the greatly increased excretion of sulfocyanate during the fourth day of dog 4 might have been due to the release of such a deposit.

Whatever the exact cause of these fluctuations in apparent volume of distribution, the fact of their existence renders questionable the equation of the apparent volume of distribution of sulfocyanate with any one fraction of the body fluids more than a few hours after injection.

The identification of the apparent volume of distribution of sulfanilamide with total body water has no experimental support whatsoever. If it is calculated on the basis of its concentration in either serum water or cell water, the results are absurdly high or low respectively. Painter's use of the concentration of sulfanilamide in whole blood water to calculate its apparent volume of distribution lacks physiological justification, since the indicated concentration is present neither in the serum phase nor in the cell phase. The results are dependent on the fortuitous combination of cell concentration, serum concentration, and cell volume. Figures so obtained may be approximately of the correct magnitude but are necessarily without physiological meaning.

Waterhouse and Shannon (11) have published experiments leading to the same conclusion. Using the concentration in serum water for the calculation they obtained apparent volumes of distribution of sulfanilamide ranging from 88 to 102 per cent of the body weight. However, they did not take into account the corrections for the non-diffusible fractions in serum and hence obtained lower values than were found in our experiment.

SUMMARY

A modification is described of the method of Crandall and Anderson for the determination of sulfocyanate in serum and urine of the dog.

Comparable values were obtained at four hours after injections of sulfocyanate in two dogs which were given three successive injections.

Uniform values were also obtained at one, two and four hours after three successive injections in two other dogs.

In three of four dogs the apparent volume of distribution of sulfocyanate increased with time over 24 to 96 hours after injection.

The data indicate that in the dog the apparent volume of distribution of sulfocyanate determined between one and four hours after injection measures a definite fraction of the body fluids which approximates the extracellular fluid volume.

The data also indicate that the apparent volume of distribution of sulfocyanate in the dog is not a valid measure of any one fraction of the body fluids after the fourth hour following injection.

Sulfanilamide was found to be unevenly distributed between serum and cells in dog blood in vivo. The distribution of sulfanilamide cannot be used to measure total body water or any fraction thereof.

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ABNORMAL CAPILLARY RESISTANCE IN SWINE SUFFERING FROM AN INHERITED BLEEDING DISEASE[1]

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During the last few years a strain of swine owned by the Missouri Agricultural Experiment Station has been under observation because of severe bleeding tendencies. Many of the animals bleed to death from trivial wounds or from apparently spontaneous hemorrhage. Several of the characteristics of the abnormality have been described: the disease is transmitted by both sexes as a Mendelian recessive (1); the blood clotting mechanism is defective (2, 3), and the saline bleeding time is abnormally prolonged (4).

The discovery of an abnormal saline bleeding time has prompted us to test the capillary resistance of the bleeder animals, for in man low capillary resistance and abnormal bleeding times often occur together (5).

Methods. Capillary resistance was measured by the Dalldorf suction method (6). Negative pressures accurate to within ±0.5 cm. of mercury were obtained with either a vacuum pump, or with a water aspirator (filter pump), connected to a mercury valve (7) and a mercury manometer. Because of frequent breakage of the glass suction cup designed by Dalldorf (6), we substituted a rubber tube with a bore of 1 cm. and a wall thickness of 8 mm. The animals were strapped down securely on their backs, and the suction tube was applied to the inside of the thigh 1 to 3 inches from the midline.

In preliminary experiments 9 purebred Poland China swine 4 to 6 months of age were tested. The 4 bleeder animals gave 5 or more petechiae when a negative pressure of 75 cm. of mercury was applied for 2 minutes. Four of the 5 normal animals, however, gave no petechiae at this maximum negative pressure. It was therefore impossible to measure quantitatively the normal capillary resistance of the dark-skinned Poland Chinas, and we conducted subsequent experiments on white, or lighter-skinned, Chester White-Poland China crosses. These animals were obtained by breeding 2 white-skinned Chester White-Poland China sows (litter mates carrying the bleeder gene (1)) to male 1, a Poland China bleeder described in a previous publication (4). A total of 11 offspring was obtained in the 2 litters. It was found that all of these closely related animals had capillary resistances that were measurable. Seven of the animals were white, and 4 were black. The white animals were devoid of pigment, and the black animals had very light skins on the inside of the thighs, so that it was easy to detect and count the petechiae. The 11 animals were used for capillary resistance tests when the group had reached the age of 4 months and the individuals weighed 72 to 95

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lbs. Male 6 died before the tests were completed. Preliminary tests, however, indicated that his capillary resistance was abnormally low. Female 11 was discarded because she was a borderline type of animal (1), *i.e.*, neither a severe bleeder nor completely normal.

Results. To secure quantitative information on the bleeding time in the animals chosen for study, saline bleeding time tests were run at weekly intervals for 3 weeks, using the technique previously described (4). The data obtained are shown in table 1. The animals F5, M4 and F1 are from one litter, the remaining animals are from the other litter. The animals fall into 2 groups: the carriers[2], with average saline bleeding times of less than 100 seconds, and the bleeders, with average saline bleeding times of more than 300 seconds.

An experiment on the relative incidence of petechiae in the carriers and bleeders was carried out in conjunction with the saline bleeding time tests. Eight of the animals were grouped into pairs of 1 carrier and 1 bleeder. The carrier animals

TABLE 1

The saline bleeding time in Chester White-Poland China swine

WEEK	CARRIERS					BLEEDERS			
	F8	F10	F5	M4	M7	F1	F9	F7	F14
	sec.	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*
1	75	120	65	120	75	600+	270	330	600+
2	90	100	65	60	60	270	600+	295	160
3	85	63	36	80	40	600+	390	600+	180+*
4	67	95	62		60	600+	600+	600+	
Average...	79	94	57	87	59	517+	465+	456+	313+

* F14 developed a lung hemorrhage 180 seconds after the saline bleeding time test had been started, and died in about 10 minutes. Autopsy showed 2 small tears on the surface of the right lung, and blood in the thoracic cavity.

F8, F10, F5 and M4 were matched respectively with the bleeders F1, F9, F7 and F14. The *highest* negative pressure that produced no petechiae in the carrier animal was determined at weekly intervals. The bleeder was then tested at the same negative pressure and the number of petechiae counted. Negative pressures of 10 to 50 cm. of mercury (at intervals of 10 cm.) were applied to the inside of the thigh for 2 minutes. The data in table 2 show that the bleeders *consistently* developed petechiae at negative pressures that did not produce petechiae in the carriers.

After the above studies had been made, capillary resistance tests based on Elliott's definition were performed on the animals shown in figure 1. According to his definition (8) capillary resistance is the *lowest* negative pressure (expressed

[2] These animals have normal saline bleeding times (4), and do not have hemorrhagic tendencies. Genetically they are not pure "normals", since they carry the recessive bleeder gene. Inasmuch as they do not suffer from the bleeding disease, they are very satisfactory controls in the present studies.

in cm. of mercury) that is capable of producing 2 or more macroscopic petechiae in a skin area 1 cm. in diameter. The negative pressure is applied for 1 minute. In our experiments, the animals were tested daily for 8 consecutive days. On days 1, 3, 5 and 8, the determinations were made in the afternoon, and on the remaining days in the forenoon. The majority of the white animals showed a daily variation in resistance. The capillary resistance was usually 10 to 15 cm. lower in the afternoon than in the morning.

Figure 1 shows that the capillary resistance, as defined by Elliott, ranged from 35 to 60 cm. in the carrier animals, and from 5 to 35 cm. in the bleeders. The

TABLE 2

Number of petechiae in the bleeder animals at the highest negative pressure that produced no petechiae in the carriers

WEEK	PETECHIAE IN:			
	F1	F9	F7	F14
	no.	*no.*	*no.*	*no.*
1	25	11	3	15
2	25	1	6	23
3	20	3	2	32
4	18	1	10	

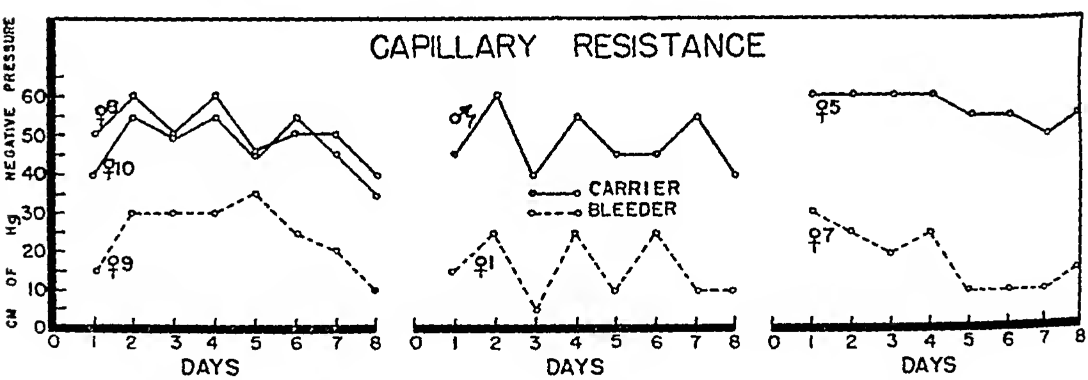

Fig. 1. A comparison of the capillary resistance of carrier and bleeder swine

average capillary resistance for the 8 day period was 51 cm. in the 4 carriers, and 19 cm. in the 3 bleeders, a difference in average resistance of 32 cm.

Discussion. Since similar methods were employed, it is possible to compare our findings (fig. 1) with resistance studies made on man by Elliott (8). He found that the capillary resistance (measured on the forearm) was in the range of 10 to 30 cm. in cases of human idiopathic thrombocytopenic purpura. The bleeder swine (fig. 1) have resistance values that fall in this same range. The carrier swine, however, have a range of capillary resistance, and an average capillary resistance, that are somewhat higher than those found in normal man (8). The differences in capillary resistance which exist between the carrier and

bleeder swine are therefore as great, or even greater, than those found by Elliott to exist between normal and purpuric humans.

SUMMARY

Evidence is presented to show that the capillary resistance is abnormally low in swine suffering from an inherited bleeding disease.

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METABOLISM OF ASPHYXIATED SPINAL CORD

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It has been found in this laboratory that when the spinal cord of a cat is asphyxiated for 55 to 65 minutes, there is a temporary return of some reflex activity (extensor tone and tendon reflexes) beginning 1½ to 4 hours after the end of asphyxiation, and lasting up to 48 hours (van Harreveld and Marmont, 1939). After this temporary return, reflex activity usually is absent for the rest of the animal's life. It seemed of interest to follow the metabolic changes of the cord which accompany the above changes. For this reason the oxygen uptake of excised spinal cord has been determined at various intervals after a 60 minute asphyxiation.

After asphyxiation of the cord for 35 minutes some reflexes return temporarily (flexion reflex), but others, such as extensor tone and tendon reflexes return permanently. It has been found (van Harreveld, 1941) that the reflex activity present 14 days after such an asphyxiation is much more resistant to oxygen lack than the reflexes of a normal animal. This increased resistance is not present 48 hours after asphyxiation, but develops slowly in the ensuing days. It was of interest, therefore, to determine also the cord metabolism 2 and 14 days after a 35 minute asphyxiation, when the resistance of reflex activity to oxygen lack is so different.

Method. The caudal part of the spinal cord of cats was asphyxiated by a method previously described (van Harreveld and Marmont, 1939). The caudal part of the dural cavity and of the spinal cord was isolated by ligating the dura at the lower thoracic level. The next day the blood circulation in that part of the cord was interrupted by forcing Ringer's solution into the isolated part of the dural cavity at a pressure higher than the blood pressure.

The oxygen uptake of the cord was determined in Warburg respirometers using Ringer-glucose medium, buffered with phosphate to pH 7.4. The caudal part of the cord was removed and divided into four parts; the 6th lumbar, 7th lumbar, 1st sacral and the remaining caudal part consisting of the 2nd and 3rd sacral and the coccygeal segments (referred to as the tail). Each of these four parts was sliced parallel to the long axis of the cord with an apparatus consisting of 10 razor blades spaced at 1.5 mm. The tissue was suspended in individual, tared vessels, and weighed before placing in a bath of 38°C. The first reading was taken 50 minutes after the cord was removed. This allowed 15 to 20 minutes for the vessels to come to temperature equilibrium. The metabolism was calculated from the oxygen uptake between 50 and 80 minutes after removal of the cord, and the results were expressed in cubic millimeters of

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oxygen consumed/100 mgm. of wet tissue/hour. The average oxygen uptake of the spinal cord caudal of L5 was calculated from the weights and oxygen consumption of the 4 parts.

In one series of experiments the parts were minced instead of sliced. To study the metabolism of white matter, a number of experiments were carried out on the optic nerve, which may be considered as central white matter.

TABLE 1

Metabolism of normal spinal cord in cubic millimeters of O_2/100 mgm. tissue/hour

The value in the last column of tables 1, 4, 5 and 6 is the O_2 uptake of the entire region of the cord caudal of L5, and is calculated from the individual weights and oxygen consumption of L6, L7, S1 and the tail.

NUMBER	L6	L7	S1	TAIL	L6-TAIL
A. Minced tissue, mature cats					
1	48	58	80	69	62
2	49	52	62	58	54
3	43	54	56	56	51
4	49	58	55	69	57
5	49	52	58	66	55
Average.......	48	55	62	64	56
B. Sliced tissue, mature cats					
6	54	65	77	96	70
7	51	66	69	90	67
8	55	55	83	101	73
9	56	61	82	89	69
10	44	54	61	95	69
Average.......	52	60	74	94	70
C. Sliced tissue, young cats					
11	59	69	72	85	71
12	67	87	86	95	81
13	62	76	87	97	79
14	69	80	86	117	86
Average.......	64	78	83	99	79

Results. A. *Normal spinal cord and optic nerve.* Three points are to be noted which influence the oxygen uptake of normal spinal cord and optic nerve.

1. *Metabolism of sliced and minced tissue.* Table 1, A and B, shows that the oxygen uptake is considerably lower in minced than in sliced spinal cord. This difference is more marked in the optic nerve (table 2). Mincing the nerve reduces its metabolism to a fraction of that of an optic nerve sliced in two, lengthwise. The optic nerve is small enough to be used without slicing; the oxygen uptake of the unsliced nerve was found to be only a little higher than that found

in sliced preparations, showing that slicing has only a small influence on tissue respiration. The values found for minced nerves are of the same order as those of Michail and Benetato (1936) who used human optic nerves. The much greater influence of mincing on the oxygen uptake of the optic nerve than on that of the spinal cord may be due to the firmer consistency of this nerve. Hence more force, causing more damage, is needed to mince the optic nerve.

Since slicing obviously causes less damage than mincing, all studies on the spinal cord were made with sliced preparations. However, since the unsliced optic nerve has the highest metabolism (table 2), this nerve was used without further preparation.

It is of interest to note that the metabolism of unsliced peripheral nerve (median nerve) averaged 15 mm.^{3} of O^2/100 mgm. tissue/hour, as compared to 45 mm.^{3} for unsliced optic nerve.

TABLE 2

Metabolism of optic nerves in cubic millimeters of O_2/100 mgm. tissue/hour

NUMBER	INTACT	SLICED IN TWO	MINCED
1	45	38	
2	48	45	
3	46	41	
4	43	41	
5	53		8
6	46		7
7	35		7
8	41		16
9		42	5
10		35	8
Average............	45	40	9

2. *Influence of age of the animal.* Table 1, C, shows that the spinal cord of young cats has a higher oxygen uptake than that of adults. The same has been found for the brains of young and adult dogs (Himwich and Fazekas, 1941) and rats (Tyler and van Harreveld, 1942). Only mature animals have been used for the asphyxiation experiments.

3. *Gradient of metabolism.* There is a very marked gradient of respiratory activity in the caudal part of the spinal cord. However, unlike the gradient found in the brain, the more cranial parts of the cord have the lowest activity, while the most caudal part has the highest activity. Tables 1 and 4 show that the oxygen uptake of the "tail" is more than $1\frac{1}{2}$ times as great as that of the 6th lumbar segment. It is therefore of the utmost importance to define, as exactly as possible, the segments on which the metabolism is determined.

B. *The effect of severing optic nerve and spinal cord.* Transection of the optic nerve directly behind the eye results in a drop of its metabolism. The respiration of the optic nerve 3, 7 and 14 days after transection was around 35 mm.^{3}/100 mgm. tissue/hour, whereas the average O^2 consumption of the heterolateral nerve, which was left intact, was 46 mm.^{3} (table 3).

Severing of the spinal cord also results in a drop of its metabolism, which, however, is slight. An oxygen uptake of 70 mm.³ was found for the unsevered cord (table 1, B); the values 1, 3, 7 and 14 days after ligating dura and cord were about 65 mm.³ (table 4). Since severing of the spinal cord is a preliminary step for cord asphyxiation, the latter values are to be considered as controls for the asphyxiated cord.

C. *The effect of asphyxiation of the cord.* 1. *Metabolism of the spinal cord after 60 minutes' asphyxiation.* In these experiments the spinal cord was removed at intervals varying from 0 hour to 14 days after 60 minutes' asphyxiation. In the 0 hour experiments, the cord circulation was not re-established, but it should be pointed out that it takes 50 minutes to prepare and equilibrate the tissues and during most of that period the sliced cord is kept in an oxygen saturated medium. Table 5 shows that the oxygen uptake of the cord, taken

TABLE 3

Metabolism of normal and degenerated optic nerves in cubic millimeters of O_2/100 mgm. tissue/hour

NUMBER	NORMAL SIDE	DEGENERATED SIDE		
		After 3 days	After 1 week	After 2 weeks
1	40	35		
2	51	35		
3	47	31		
4	52		33	
5	34		33	
6	55		49	
7	48			33
8	42			27
9	48			34
10	41			34
Average........	46	34	38	32

out immediately at the end of asphyxiation, is 60 mm.³ or almost as high as that of the normal isolated cord. The metabolism of the cords removed after intervals of 3, 6 and 12 hours shows a progressing decline. The oxygen uptake of the cord after these various intervals was respectively 56, 48 and 38 mm.³ After an interval of 48 hours, the low level of about 30 mm.³ is reached which is maintained during the next 14 days. This level is about half of that of the normal isolated cord. It can be concluded that the full damaging effect of 60 minutes' asphyxiation on oxygen uptake is not apparent immediately, but develops in the ensuing 12 to 48 hours.

2. *The metabolism of the spinal cord after 35 minutes' asphyxiation.* The metabolism 2 days after 35 minutes' asphyxiation showed an average of 41 mm³.; after 14 days the oxygen uptake had decreased to an average of 31 mm³ (table 6). The oxygen consumption found 14 days after 35 minutes' asphyxiation was not significantly different from that found 2 weeks after 60 minutes' asphyxiation,

though the 35 minute animals, contrary to the 60 minute ones, showed some reflex activity (usually extensor tone).

Anatomical changes in the cord. The 5th lumbar segment, adjacent to the part of the cord that had been used for metabolism determination, was fixed in 95 per cent alcohol, sectioned and stained with toluidine blue. These preparations showed changes similar to those described, for instance, by Tureen (1934) in asphyxiated spinal cord. The cord removed immediately after a 60 minute asphyxiation showed a normal appearance. After 3 hours considerable chromatolysis was present, some cells stained poorly, and in many cells the

TABLE 4

Metabolism of severed spinal cord in cubic millimeters of O_2/100 mgm. tissue/hour

NUMBER	INTERVAL AFTER TRANSECTION	L6	L7	S1	TAIL	L6-TAIL
1	1 day	49	70	66	82	68
2		47	55	64	81	60
3		59	65	73	76	66
Average.............		52	63	68	80	65
4	3 days	53	55	60	79	61
5		60	77	72	87	70
6		50	52	70	84	62
Average.............		54	61	67	83	64
7	1 week	46	59	62	75	60
8		44	53	56	84	60
9		54	64	70	84	67
Average.............		48	59	63	81	62
10	2 weeks	52	56	62	81	62
11		46	62	62	95	67
12		65	67	76	85	72
Average.............		54	62	67	87	67

nuclear boundary was not distinct. After 6, and especially after 12 hours, a marked decrease of the number of ganglion cells was apparent. Chromatolysis was about complete and most cells were poorly stained. Infrequent neurophagia was observed. After 48 hours no ganglion cells could be found. There was a slight increase in the interstitital cells, and usually a proliferation of the capillaries. After 7 and 14 days, a considerable increase of the interstitial cells was observed. Most of these cells had a pale, vesicular nucleus; some polymorphonuclear cells were present.

Preparations of the cords removed 2 days after 35 minutes' asphyxiation showed a greatly reduced number of ganglion cells with advanced chromatol-

TABLE 5

Metabolism of spinal cord asphyxiated for 60 minutes in cubic millimeters of O_2*/100 mgm. tissue/hour*

NUMBER	INTERVAL AFTER ASPHYXIATION	L6	L7	S1	TAIL	L6-TAIL
1	0 hour	47	56	68	85	63
2		50	63	66	71	61
3		48	59	62	72	59
4		48	63	58	70	57
Average		48	60	64	75	60
5	3 hours	53	61	57	79	62
6		33	42	43	75	47
7		42	57	65	94	62
8		41	49	61	61	51
Average		42	52	57	77	56
9	6 hours	40	37	31	44	39
10		33	47	55	79	55
11		38	49	63	77	55
12		33	42	45	56	44
Average		36	44	49	64	48
13	12 hours	40	41	45	53	45
14		23	26	38	47	32
15		27	33	35	47	35
16		38	33	41	51	39
Average		32	33	40	50	38
17	2 days	31	28	38	46	36
18		23	24	37	37	29
19		27	26	39	33	31
20		23	22	21	31	24
Average		26	25	34	37	30
21	1 week	29	29	46	52	37
22		34	35	34	32	34
23		23	24	28	35	27
24		25	28	38	35	30
Average		28	29	37	39	32
25	2 weeks	23	18	20	25	22
26		23	18	20	52	30
27		36	30	25	43	31
28		22	25	37	43	31
Average		26	23	26	41	29

ysis. Furthermore, there was increase of the interstitial cells and proliferation of the capillaries. Two weeks after 35 minutes' asphyxiation, the ganglion cells showed regeneration of Nissl substance; about the same number of nerve cells, as found at 48 hours, was present. The same increase of interstitial cells, as found 2 weeks after 60 minutes' asphyxiation, was observed.

A slice of the 5th lumbar segment of the cats surviving 35 or 60 minutes' asphyxiation for 2 weeks, was stained with the Marchi method. Heavy degeneration was found in the anterior and lateral columns; the degeneration in the posterior columns, however, was only slight. It was found previously (van Harreveld, 1940) that 65 minutes' asphyxiation of the cord leaves the

TABLE 6

Metabolism of spinal cord asphyxiated for 35 minutes in cubic millimeters of O_2/100 mgm. tissue/hour

NUMBER	INTERVAL AFTER ASPHYXIATION	L6	L7	S1	TAIL	L6-TAIL
1	2 days	20	21	26	52	30
2		32	32	39	82	46
3		28	34	42	52	37
4		28	33	41	63	37
5		34	35	44	72	45
6		44	47	61	64	53
Average..............		31	34	42	64	41
7	2 weeks	19	23	33	60	34
8		34	35	45	54	41
9		17	24	27	38	25
10		25	34	39	39	33
11		17	21	27	41	26
12		20	20	25	41	25
Average..............		22	26	33	46	31

posterior roots intact, which explains the slight degeneration in the posterior columns.

Discussion. The gradient of metabolic activity found in the normal spinal cord is of interest particularly since it is of the reverse order than that in the brain. This gradient is probably due to the relative amounts of gray and white matter present in the different segments of the cord. The gray matter has a higher respiration rate than the white matter (Holmes, 1930), and it has been shown by Donaldson and Davis (1903) that the relative amount of gray matter in the caudal part of the human spinal cord increases considerably from the cranial to the caudal segments. This increase seems to be great enough to account for the gradient in metabolism.

The Wallerian degeneration of the nerve fibers with disintegration of the myelin sheaths probably causes the drop in metabolism after transection of the

optic nerve. The slight drop in metabolism after transection of the cord may be caused by the degeneration of the descending tracts. The destruction of ganglion cells and the secondary degeneration of their fibers after asphyxiation of the cord will obviously cause a much more marked drop in the oxgen uptake.[2] Although the interstitial cells probably have a low metabolism, their marked increase 1 to 2 weeks after asphyxiation may offset, to a certain extent, the drop due to destruction of ganglion cells and nerve figers.

It was found that the oxygen uptake of the cord returns to almost normal values shortly after a 60 minute asphyxiation, but decreases in the ensuing 12 hours to a low level. This may indicate that for a short period after 60 minutes' asphyxiation, the concentration of the respiratory enzymes is approximately normal. However, since the enzymes cannot be replaced by the structures damaged by asphyxiation, respiration and other functional activities due to these enzymes soon become lost. Parallel with these metabolic changes, reflex activity returns after such periods of asphyxiation, to disappear again a number of hours later (van Harreveld and Marmont, 1939).

The possibility has been considered (van Harreveld, 1941) that the high resistance of reflex activity to oxygen lack shown by cats 14 days after a 35 minute asphyxiation might be due to a very low cord metabolism in these animals, enabling the tissue to function for an unusually long period on the small amount of oxygen present in blood and tissue. At that time it had been argued that this explanation is unlikely, and the present experiments substantiate this view. The difference between the oxygen uptake 48 hours after a 35 minute asphyxiation (when the high resistance to oxygen lack has not yet developed), and after 14 days, is not large, whereas the survival time of the reflex activity at 14 days is many times greater than that at 48 hours.

SUMMARY

The oxygen consumption of the caudal part of the normal and asphyxiated spinal cord and of the optic nerve was determined by means of Warburg respirometers. It was found that:

1. A gradient of oxygen consumption was present in the region of the cord studied (caudal of L5). The most caudal part had the highest metabolism. This gradient is the reverse of that found in the brain.

2. Wallerian degeneration of the optie nerve and of descending tracts in the spinal cord results in only a moderate decrease of the oxygen uptake.

3. Destruction of the perikarya, with secondary degeneration of the rest of the neurons, by 60 minutes' asphyxiation, causes a marked decrease in oxygen uptake.

4. When the cord is examined immediately after a 60 minute asphyxiation, the metabolism is only slightly lower than in the normal cord. In the ensuing 12 to 48 hours the oxygen uptake drops to about half the value found in the

[2] An increased water content of the optic nerve and cord due to transection (circulatory disturbances) or asphyxiation (capillary damage) may, during the first days, cause additional depression of the metabolism.

unasphyxiated cord. During the rest of the period of observation (two weeks) the metabolism remains at this level.

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PHOSPHORUS ABSORPTION IN THE ANESTHETIZED DOG AS DETERMINED WITH THE RADIOACTIVE ISOTOPE[1]

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Few measurements are recorded of radioactive phosphorus in the blood and liver soon after the administration of the isotope, i.e., during the period of maximum absorption. Furthermore, since rats and mice have been used almost exclusively for this type of investigation, the reported changes in the P* content of tissues with time are based on values obtained from animals which were sacrificed at the time of sampling. The use of the dog as the experimental animal in this study had the following advantages: 1, samples of liver and of arterial, portal and jugular blood were taken simultaneously; 2, the changes in the isotope content of these tissues were observed on the same animal at different intervals of time.

METHODS. The P* was given as sodium phosphate dissolved in 100 cc. of 0.9 per cent NaCl at pH 6 to 7. Doses, ranging from 542,000 to 4,250,000 counts per minute (scale-of-four Geiger-Müller counters), were introduced through a glass cannula into the duodenum of anesthetized[2] dogs (11 to 23 kgm.). No phosphorus determinations were made since the resultant sacrifice of P* would have been considerable, but the dose probably never exceeded 15 mgm. of phosphorus.

Samples of blood from the portal, jugular and carotid vessels, of liver, and of intestinal contents were taken at intervals up to 4 hours each, and the material weighed as soon as possible. V-shaped pieces were cut from the edge of the liver and the wounds thus made closed immediately with gut sutures. Intestinal contents were removed through the intestinal wall with a hypodermic needle. When a urine sample was desired, the bladder was washed out with saline and the washings added to the urine which had been excreted during the period. At the end of the experiment the small intestine was removed and washed out thrice with saline and an aliquot of the washings taken for the P* determination. The liver was removed and weighed to permit a calculation of the total P* present in the organ at any time. One dog had a Thiry-Vella fistula which was washed out with saline at hourly intervals, and the activity of the washings determined. Samples of muscle (biceps femoris) were taken at various intervals.

The samples were dissolved in fuming nitric acid, and the radioactivity of the acid solution measured by the technique of Bale, Haven and LeFevre (1). Results are expressed as the percentages of the original dose per gram of blood, liver, skeletal muscle, and intestinal contents; the values for the urine, and the intestinal and fistula washings, are in terms of the total isotope present.

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[2] Dial with urethane kindly supplied by the Ciba Pharmaceutical Company.

RESULTS. The mean percentages of the dose $\times 10^{-3}$ per gram of tissue and the specific activities of the tissues are given in table 1. Since determinations of arterial blood and intestinal tissue were made in only two of the eight dogs studied, the values obtained on these two dogs are given separately in table 2. The values for total phosphorus content of blood and liver were taken from Greenwald (2) and Flock, Bollmann and Mann (3), respectively.

Blood. A significant amount of P*[3] appeared in the arterial, portal and jugular blood 5 minutes after administration of the isotope and, as table 1 shows, the

TABLE 1

P in the blood and liver during absorption from the intestine*

	TIME (IN MINUTES)										
	5	15	30	45	60	90	120	150	180	210	240
	Number of experiments										
	6	7	7	7	7	7	7	7	7	7	7
Portal blood											
10^{-3} per cent dose/gram...	1.89	4.24	4.94	5.22	5.63	5.00	5.65	5.68	5.97	5.45	5.70
10^{-3} per cent dose/mgm.P*..	4.61	10.34	12.05	12.73	13.73	12.20	13.78	13.85	14.56	13.29	13.90
	Number of experiments										
	6	7	7	7	7	7	7	7	7	7	7
Jugular blood											
10^{-3} per cent dose/gram...	0.71	1.89	2.77	3.38	3.86	4.16	4.46	4.58	5.28	4.80	5.48
10^{-3} per cent dose/mgm.P*..	1.73	4.61	6.76	8.24	9.41	10.15	10.88	11.17	12.88	11.71	13.37
	Number of experiments										
		5		5		5		5		4	3
Liver											
10^{-3} per cent dose/gram...		3.58		9.41		19.55		28.67		33.83	33.40
10^{-3} per cent dose/mgm.P†..		1.37		3.60		7.46		10.94		12.91	12.75

* Total P in blood = 0.41 mgm./gram.
† Total P in liver = 2.62 mgm./gram.

greatest change occurred within a half-hour, after which there was only a gradual increase for the duration of the experiment (4 hrs.). The average jugular blood content of P* per milligram of total phosphorus was always less than that of the portal, but this difference became less progressively and was not significant after $3\frac{1}{2}$ hours. As table 2 shows, there is no significant difference in the P* content of the jugular and carotid blood at any of the times studied.

Liver. At the earliest time of measurement, 15 minutes after P* administra-

[3] In most of these experiments a count of 3 times background, which is considered significant, represents approximately 1×10^{-5} per cent of the dose.

tion, the liver contained a considerable amount of the isotope (3.6×10^{-3} per cent of the dose/gram). The P* content increased rather rapidly for about $2\frac{1}{2}$ hours after which it maintained a fair constancy. The liver greatly exceeds the blood in the amount of P* which it acquires per gram of tissue (table 1). However, per milligram of phosphorus, the liver contains less isotope than the jugular blood for the first hour, and approximates the value for the portal blood after $2\frac{1}{2}$ hours. A comparable relationship has been shown for bone and blood by Manly, Hodge and Manly (4).

TABLE 2

P in the blood and liver during absorption from the intestine*

(2 experiments)

	TIME (IN MINUTES)									
	15	30	45	60	90	120	150	180	210	240
Portal blood										
10^{-3} per cent dose/gram	2.25	2.96	3.62	6.03	5.00	5.38	4.19	5.49	4.81	4.52
10^{-3}per cent dose/mgm.P	5.49	7.22	8.83	14.71	12.20	13.12	10.02	13.39	11.73	11.02
Jugular blood										
10^{-3} per cent dose/gram..........	0.88	1.59	2.76	3.67	3.85	4.11	4.30	4.49	4.39	4.53
10^{-3} per cent dose/mgm.P.	2.15	3.88	6.73	8.95	9.39	10.02	10.49	10.95	10.71	11.05
Arterial blood										
10^{-3} per cent dose/gram..........	1.37	1.89	3.05	3.66	4.12	3.94	4.10	4.39	4.58	4.52
10^{-3} per cent dose/mgm.P........	3.34	4.61	7.44	8.93	10.05	9.61	10.00	10.71	11.17	11.02
Liver										
10^{-3} per cent dose/gram..........	3.07		8.06		10.61		36.39		40.51	35.65
10^{-3} per cent dose/mgm.P........	1.17		3.08		4.05		13.89		15.46	13.99

A calculation based on the average percentage of the dose per gram and the average liver weights of four dogs showed that at $3\frac{1}{2}$ hours the liver contained about 15 per cent of the dose.

Intestinal contents. Since all but the final volumes were unknown, the P* determinations on intestinal contents gave only the concentration of the isotope. There were some fluctuations in concentration which appear to be fairly well correlated with the portal-jugular difference in concentration of P* (fig. 1). The secondary rise in the portal-jugular difference, which occurs between the

second and third hour, corresponds with the secondary rise in the concentration of the isotope in the intestinal contents at about the same time. Russell and Nasset (5), who fed a test meal to dogs bearing jejunal fistuli, observed a similar secondary rise in the volume of the fistula contents occurring between the second and third hour after feeding.

Cohn and Greenberg (6) showed that P* injected intraperitoneally appeared in the feces and small intestine of the rat. In the present study, the transfer of P* from the bloodstream to the lumen of the gut was demonstrated in a dog

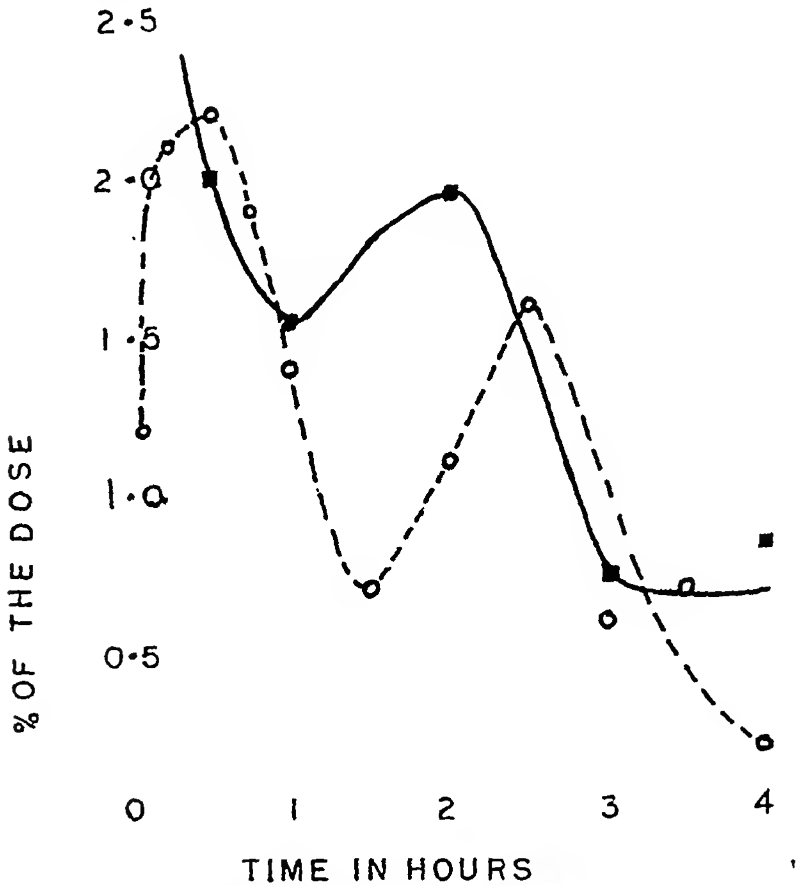

Fig. 1. ○ Average portal-jugular difference × 10^{-3} ■ per cent original dose × 10/gram intestinal contents.

bearing a Thiry-Vella fistula of the jejunum. The fistula had been established about 3 months before the animal was used for the P* absorption experiment and there was no trace of blood in the contents obtained from the fistula. It is concluded, therefore, that the P* found was secreted into the lumen as part of the normal succus entericus. The amounts of P* recovered from the Thiry-Vella fistula in four 60-minute periods were: 4.5, 2.3, 1.8, and 0.9 × 10^{-3} per cent of the dose, respectively. This indication of a steady decline in secretion of succus entericus is consistent with what is known regarding the response of the small intestine to mechanical stimulation (Nasset, Pierce and Murlin, 7).

From 0.4 to 5 per cent of the P* dose was washed out of the intestine at the end of the experiment, the greater part of which, doubtless, was unabsorbed P*, but certainly some was secreted into the intestine. In one experiment in which the pancreatic and biliary ducts were cannulated, P* was found in the pancreatic juice and the bile 45 minutes after administration of the isotope.

Urine. Since the variation was large, little can be said about our observations of the urinary excretion of the isotope: e.g., for the fourth hour, the values ranged from 3×10^{-3} to 3 per cent of the dose. The data are very scanty, but there appeared to be a correlation between the volume of urine and the percentage of the dose excreted.

In two experiments in which the urine was collected every fifteen minutes for the first hour, P* first appeared at 45 minutes in one and at one hour in the other.

Muscle. The biceps femoris muscle at 4 hours contained P* in amounts ranging from 0.7 to 3.5×10^{-3} per cent of the dose per gram of tissue.

Discussion. Under our experimental conditions the absorption of inorganic phosphate appeared to be very rapid; in all instances, 5 minutes after administration there was significant amount of P* in the general circulation As expected, the portal always contained more P* than the jugular blood. The liver, which averaged 2.4 per cent of the body weight, removed about 1 per cent of the dose from the circulation in 15 minutes and over 15 per cent in $3\frac{1}{2}$ hours. At the end of 4 hours, only 5 per cent of the dose could be washed out of the gut; so 95 per cent had disappeared from the intestinal lumen. At this time the liver contained 15 per cent, the blood 9 per cent, and, if we assume that the P* content of the biceps femoris is representative, the skeletal muscle about 13 per cent. According to the data of Volker and Sognnaes (8) who measured the radioactivity of the bone of these dogs, the skeleton accounted for about 10 per cent and the total dentition about 0.12 per cent. The sum of these figures plus the maximum excretion in the urine (14 per cent) adds up to about $\frac{2}{3}$ of the dose. The other viscera which were not studied could be expected to contain only a small part of the missing 30 per cent of the dose which had disappeared from the gut lumen; so we suspected that a considerable amount of the marked phosphorus was held in the intestinal tissue—probably the mucosa. Other calculations based upon the portal blood flow and the dilution in the intestine required for the observed removal of P* from the gut lumen pointed in the same direction.

According to Grab, Janssen and Rein (9), the average portal blood flow in anesthetized dogs is about 0.5 cc. per gram of liver per minute. In the first thirty minutes of our experiments, therefore, the portal flow was 5 to 7 liters (table 3). If we assume that the portal blood discharged all of its P* before returning, the removal of P* from the lumen of the intestine as indicated by the analysis of the contents would require a portal flow of 15 to 18 liters in 30 minutes. The arterial blood supplying the intestine is not free of P*, and hence the contribution from the intestine is something less than the amount indicated by the P* content of the portal blood.

If the portal-jugular difference be taken as an index of the absorption from the

gut, the portal blood flow necessary to remove the required amount of P* from the lumen is increased 4 to 10 fold. Dilution in the intestine plus absorption could account for some of the change in concentration of P* in the lumen. As introduced the concentration was 1.0 per cent/gram. In thirty minutes, it was reduced to between 0.05 and 0.15 per cent/gram. If an average portal blood flow is assumed and the portal value for P* is taken as a measure of absorption, the dilution required in the intestine is 4 to 14 times the original. The intestine was never extended with fluid as one might expect even with only 400 ml. in the lumen. Using the portal-jugular difference as a measure of P* absorption, of course, increases the dilution required. These discrepancies between the portal blood flow which could be reasonably assumed, and that which could be calculated on the basis of the P* content of the blood and intestinal contents

TABLE 3

Calculations based on analyses made 30 minutes after P administration*

	DOG NUMBER		
	3	4	5
Weight (kgm.)	19.1	17.3	13.7
$P^* \times 10^{-3}$ portal blood	4.80	6.29	6.47
Portal-jugular difference	1.62	1.51	4.19
Liver weight	466	393	331
Portal blood flow			
Calc. liters in 30 min.*	7.0	5.9	5.0
Required for portal conc.†P*	17.7	14.3	14.7
Required for portal-jugular difference	52.2	59.6	22.7
Intestinal contents			
Found per cent dose/gram	0.149	0.102	0.047
Dilution required for portal P*	4.4	6.2	14.4
Dilution required for portal-jugular difference	6.0	9.0	17.0

* Average value for portal blood flow to liver—0.5 cc./gram liver/min. (Grab, Janssen, and Rein, 1929).

† Assumption made that P* was not carried away by blood stream remained in lumen of gut.

strengthened our conclusion that considerable amounts of P* must be held in the intestinal mucosa.

In two additional dogs which were studied under comparable conditions, the intestine was removed from the point of cannulation to the junction with the large bowel; samples of mucosa, muscle, and whole tissue were taken for radioactivity determinations. The intestine as a whole was thus found to contain about 20 per cent of the dose, $\frac{9}{10}$ of which was held in the mucosa. At the end of 4 hours, therefore, $\frac{1}{5}$ of the administered dose is held in the intestinal tissue, principally the mucosa. Our data give us no indication of whether the phosphorus is simply trapped in the mucosa in the form in which it was given, inorganic phosphate, or whether it has replaced unlabeled phosphorus and become an integral part of the tissue.

SUMMARY

1. A study was made of the distribution of radioactive phosphorus, introduced directly into the duodenum of the dog, during the four hours immediately following the administration of the isotope.

2. Labeled phosphorus appeared in the general circulation within five minutes. The maximum change in the P* content of the portal and jugular blood occurred within the first half-hour; the portal P* level exceeded that of the jugular and carotid blood significantly for 3 to $3\frac{1}{2}$ hours.

3. The specific activity of the liver rose rapidly for $2\frac{1}{2}$ hours, after which it remained fairly constant.

4. At the end of 4 hours, between 0.4 and 5 per cent of the dose could be washed out of the small intestine and the intestinal tissue was found to contain 20 per cent of the dose—$\frac{9}{10}$ of which was in the mucosa.

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AN EXPERIMENTAL STUDY OF THE TOURNIQUET AS A METHOD FOR INDUCING CIRCULATORY FAILURE IN THE DOG

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Tourniquets have long been used for temporary control of hemorrhage from vessels of the extremities, but only as an emergency measure, for it is well known that their subsequent release may lead to fatal shock. During World War I it was considered probable that the circulatory failure so induced was due to the absorption of toxic metabolites into the general circulation. This work has been adequately reviewed by Cannon (1). More recently several investigators have studied this shock inducing procedure (2–6)[2], without, however, reaching unanimous agreement as to the basic changes which lead to the circulatory collapse. Our attention was directed to a study of the tourniquet method for producing shock because of its apparent simplicity and the ease with which it could be quantitated.

Methods employed. Healthy, vigorous dogs weighing 8 to 20 kgm. were used. They were anesthetized with Nembutal (0.5 cc. per kgm. body weight, given intravenously), the hair carefully removed from both hind legs, and a heavy walled rubber tubing (diameter 120 mm.) was looped three times around each hip, as high as possible and tied very tightly. At the end of five hours the tourniquets were released.

Hematocrit determinations were made in capillary tubes centrifuged at 20,000 RPM in an air turbine; hemoglobin by the method of Newcomer; serum proteins by the falling drop method of Barbour and Hamilton (7); plasma volume by the T-1824 dye method of Gregersen and Stewart (8), using a spectrophotometer; blood pressure by the intra-arterial needle puncture method (9). Since both hind legs were involved in the experiment, the femoral artery usually was not available for frequent pressure recordings. Where pressure changes were believed critical, the brachial artery was exposed for this purpose. This was not adopted as a routine procedure because of the added trauma involved.

In these studies plasma volume changes, as measured by repeated dye injections, and as calculated on the basis of hematocrit and hemoglobin values proved to be of the same order of magnitude. For continuous records, the hematocrit, the simplest of the measurements, was used as the gauge of circulating plasma volume changes. The agreement between hematocrit change and alteration of

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[2] Best and co-workers of Toronto University, and Winternitz of Yale University have also studied this problem, using dogs as experimental material (personal communication).

plasma volume was checked at intervals on a selected number of animals from each series by the direct dye injection method.

Anesthesia was not maintained longer than 24 hours. Ninety-six per cent of our untreated animals subjected to a 5 hour constriction of both legs died in shock. A lesser interval of constriction gave variable results, e.g., 50 per cent of the dogs survived a constriction of both legs for 3.5 hours, and none developed circulatory failure following release of the tubes for 2 hours. Substitution of rubber blood pressure cuffs inflated to 300 mm. Hg pressure for 5 hours proved fatal in only 50 per cent of the cases.

Symptoms, hemoconcentration, etc., during the 5 hour constriction. Blood pressure, pulse and respiration remained essentially unchanged throughout the interval during which the tourniquets were in place (table 2). There was, during this period, a progressive diminution in circulating plasma volume, as indicated by hematocrit, hemoglobin, serum protein, and plasma volume changes (table 1). Plasma volume determinations made prior to and after the placing of the tourniquets indicated that from 11 to 16 per cent of the original plasma volume had been trapped in the legs below the constrictions. For the calculation of plasma volume changes during the constriction period, therefore, hematocrit, hemoglobin and serum protein concentrations were corrected by 12 per cent. These calculations would indicate an average plasma volume reduction of some 10 per cent during the interval in which the tourniquets were in place. The greatest part of the plasma volume reduction occurs in the first 1 to 2 hours, with but slow decline thereafter. Amputation of the leg beyond the constrictors showed no observable leakage of blood. Therefore the plasma loss is seemingly due to a generalized transudation.

TABLE 1

Blood concentration changes in dogs subjected to a five hour period of constriction of both hind legs

	NO. OF DOGS	HEMATOCRIT	NO. OF DOGS	HEMOGLOBIN	NO. OF DOGS	SERUM PROTEIN	NO. OF DOGS	PLASMA VOLUME
		per cent		*grams per cent*		*grams per cent*		*cc./kgm.*
Initial	40	38.3 ±0.5	40	15.1 ±0.3	32	5.7 ±0.1	12	49.7 ±0.8
2.5 hours	11	47.4 ±1.7	11	18.5 ±0.7	11	6.6 ±0.2	6	46.3 ±0.9
5 hours	40	48.8 ±0.7	40	19.5 ±0.5	32	6.5 ±0.3	12	44.3 ±0.8
Release of constrictions								
6 hours	22	64.2 ±1.5	24	28.0 ±0.6	18	7.6 ±0.3	8	30.4 ±1.5
8 hours	19	69.7 ±1.3	22	29.6 ±0.7	15	7.2 ±0.3	6	26.0 ±1.6
11 hours	10	70.9 ±1.7	14	29.5 ±0.6	11	7.1 ±0.2	6	25.4 ±1.3

When the constrictors were removed, the immediate swelling of the legs indicated a rapid loss in plasma volume through the damaged capillaries. The hematocrit level rose rapidly to 60 to 68 per cent during the first hour, then more gradually for the next 2 to 3 hours, reaching a plateau at an average of 70.9

per cent (table 1). In the same interval, hemoglobin readings rose to 29.5 grams per cent, the blood coagulation time fell from an initial of about 6 minutes to from 1 to 2 minutes, and the plasma volume (T-1824) declined to 25.4 cc. per kgm. body weight. These values remained essentially unchanged until the death of the animal. In other words, of the total plasma volume reduction of 49 per cent during the application of the constrictors and their release, roughly a fourth was lost before untying the constrictions, a half was lost in the first hour after their release, and the final fourth in the following 2 to 3 hours.

Serum protein concentrations did not show changes of the order expected from the hematocrit, hemoglobin, and plasma volume values. A rise to 7.6 grams per cent followed the release of the constriction, but there was usually no further increase as the blood concentration became more severe. In fact, almost all animals showed a decline some time before death. This probably indicates a heavy loss of plasma protein into the extra-vascular regions of the injured legs greater than that which could be returned by the lymph channels. The clear yellow fluid expressed from the leg at death showed protein concentrations approximating that of plasma. Peritoneal fluid also showed a high protein content.

The extreme and rapid concentration of the blood immediately following the release of the constrictions was not necessarily accompanied by changes in clinical signs. Deep shock followed the removal by 3 to 4 hours, as judged by decline in skin temperature, pallid lips and a lessening of the need for anesthesia. The blood pressure declined to about 90 mm. Hg shortly after removal of the constrictions, but then fell only gradually to 75 or 80 mm. Hg, where it remained until shortly before death (table 2). Blood pressure changes therefore gave little clue as to the severity of the shock or of the approach of death. The pulse rate remained high, and was extremely variable, until a few hours before death, when there sometimes was a decline. The veins collapsed relatively early. About an hour before death, rapid gasping respirations were usually observed, and the heart beat became extremely irregular.

The survival periods of 25 dogs ranged from 3 to 27 hours after the release of the constrictions. One dog recovered (table 2). No correlation has been observed between the degree or the rapidity of hemoconcentration and the survival period of the animal.

Autopsy findings in untreated animals. Autopsy revealed few distinctive abnormalities. There was a slight congestion of the intestine but no evidence of hemorrhage; some congestion of the spleen, kidneys and liver; marked contraction of the spleen with raised areas on its surface; minor hemorrhages, sometimes widespread, into the adrenal cortex. Very little fluid was present in the peritoneal cavity.

In the earlier experiments about 60 per cent of the animals developed subcutaneous gas pockets in the tissues of the hind legs, and crepitation was felt. When the experimental technique was modified so that the legs were carefully washed and treated frequently with alcohol, during the constriction period, these infections became rare.

The hind limbs were swollen and filled with fluid which could be occasionally

expressed as either a straw colored or red tinged plasma like material. This was especially noticeable when transfusions were given immediately following release of the tourniquets.

Effect of bandaging the limbs following release of the tourniquets. The experimental evidence strongly indicated that the cause of death following release of the constrictions had origin in the local loss of fluid into the injured legs. An effort was therefore made to curtail this fluid loss by bandaging the legs snugly from thigh to toe with several layers of sterile gauze, followed by a tight wrapping

TABLE 2

The effect of leg bandaging and of saline and plasma injections in the prevention of shock after a five hour constriction of both hind legs in the dog

NO. OF DOGS	BODY WEIGHT	INITIAL		END OF CONSTRICTION		4-8 HOURS "SHOCK"		SURVIVAL	PER CENT OF DOGS SURVIVING
		Blood pressure	Pulse per minute	Blood pressure	Pulse per minute	Blood pressure	Pulse per minute		
Untreated controls									
	kgm.	*mm. Hg*		*mm. Hg*		*mm. Hg*		*hours*	
25	12.8	111 ±1.4	128 ±4.0	108 ±1.5	163 ±2.4	78 ±1.5	175 ±2.2	8 ±0.7	4
Injured legs bandaged at the end of the constriction period									
22	12.5	114 ±1.3	132 ±3.6	113 ±1.3	169 ±2.2	110 ±1.3	172 ±2.0		100
Saline infusion (300 and 900 cc.) following release of constrictions									
2	9.8	118	111	124	157	77	190	12	0
Plasma transfusion (25 cc./kgm.) given immediately upon release of the constrictions									
6	10.2	115 ±1.6	143 ±3.2	102 ±2.0	164 ±3.0	83 ±1.6	148 ±2.5	8 ±0.5	17
Plasma transfusion (25 cc./kgm.) given 3 hours after release of the constrictions									
6	10.3	118 ±1.4	140 ±3.0	95 ±2.0	162 ±2.4	75 ±1.4	151 ±3.0	12 ±1.0	33
Plasma transfusion (25 cc./kgm.) given in 5 equal doses: at time of release of constrictions, and at 1, 2, 4 and 7 hours later									
7	10.6	112 ±1.6	111 ±3.6	106 ±1.5	162 ±2.3	105 ±1.1	174 ±2.0		100

with adhesive tape. Despite the tapes, some hemoconcentration still occurred (fig. 1). The average plasma volume reduction over that reached in the constriction period was 10 cc. per kgm. body weight, or 20 per cent (fig. 1).

The animals with bandaged legs exhibited no signs of shock. They were alert from the time they were allowed to recover from the anesthetic (24 hrs.) and took food and water at that time (table 2). However, the hemoconcentration was relieved slowly, especially before water was taken (fig. 1). After 48 to 72 hours the hematocrit and hemoglobin concentrations stabilized at a

level usually somewhat higher than the initial, pre-experimental, one which was maintained until the wrappings were removed. These results are somewhat comparable to those of Duncan and Blalock (10).

Despite the fact that the bandaged dogs presented no evidence whatever of shock, any attempt to remove the bandages before 24 hours was followed by circulatory collapse. The limbs swelled, the blood concentrated and the animals

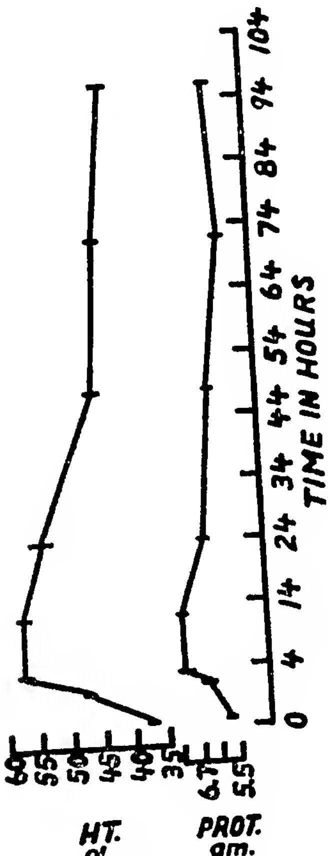

Fig. 1

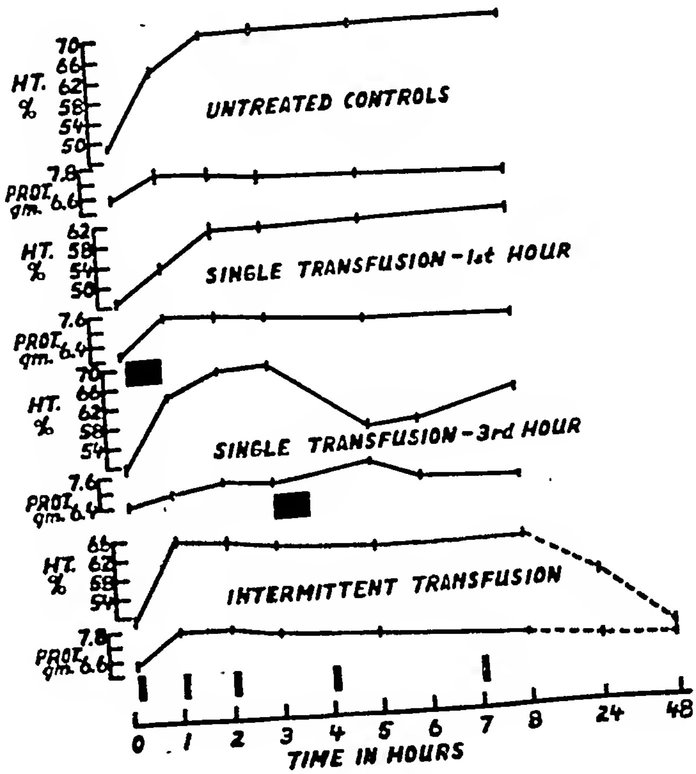

Fig. 2

Fig. 1. Blood concentration and dilution changes following release of the constrictions (zero time), while the injured legs are enclosed in tight bandages.

Fig. 2. Hematocrit and serum protein changes following plasma transfusions after a five hour constriction of both hind legs. Blocks represent amount and time of transfusions.

Single transfusion: 25 cc. heparinized plasma per kilogram of body weight.

Intermittent transfusion: Five injections of 5 cc. per kilogram of body weight each, heparinized plasma.

presented the same symptoms as control animals did when the constrictions were released. The bandages could usually be removed after 24 hours without jeopardizing the life of the animal (table 3). However, some concentration followed in practically all cases.

During the period in which the bandages were in place, blood flow in the injured limb seemed well established. At least, the dye T-1824 injected into the femoral artery or saphenous vein could be recovered in the jugular or fore limb

veins within 30 seconds. Although circulatory failure was prevented by bandaging the legs for at least 24 hours after release of the constrictions, the actual recovery of the legs was a slow process. Paralysis was present over several weeks. Necrotic areas appeared in the region where the constrictions had been placed with an eventual skin sloughing and raw ulcers. Severe infection often developed unless appropriate precautionary measures were taken.

TABLE 3

Blood concentration changes following removal of the bandages from the injured legs

DOG	BANDAGES REMOVED	INITIAL			PRIOR BANDAGE REMOVAL			5 HRS. AFTER REMOVAL			24 HRS. AFTER REMOVAL			SURVIVAL HOURS
		Hematocrit	Hemoglobin	Serum protein	Hematocrit	Hemoglobin	Serum protein	Hematocrit	Hemoglobin	Serum protein	Hematocrit	Hemoglobin	Serum protein	
	days	*per cent*	*grams per cent*	*grams per cent*	*per cent*	*grams per cent*	*grams per cent*	*per cent*	*grams per cent*	*grams per cent*	*per cent*	*grams per cent*	*grams per cent*	
1	½	47.9	19.8	6.8	67.2	28.8	7.2	80.0	33.0	7.0				5
2	½	52.0	23.4	6.4	63.3	30.4	7.4	81.6	33.0	8.7				4
3	1	42.5	19.5	6.5	63.8	28.8	7.4	65.1	29.9	6.8	58.4	27.4	5.1	Recovered
4	1	32.4	15.2	5.2	50.3	21.7	6.2	56.0	29.9	6.8	51.4	20.9	5.3	Recovered
5	2	41.8	15.1	6.8	49.1	17.3	7.1	54.0	19.0	7.3				5
6	2	35.5	11.2	6.2	40.0	13.3	6.9	49.0	16.0	7.4				10
7	2	40.3	15.7	4.9	49.2	23.8	6.1	54.1	24.5	7.8	47.5	20.5	4.3	Recovered
8	2	43.2	16.0	6.1	50.0	24.0	6.4	48.0	21.4	6.1	47.5	21.6	5.9	Recovered
9	3	41.0	14.2	5.4	44.6	18.9	7.8	62.8	21.2	7.9				12
10	3	29.6	11.7	5.3	40.4	14.2	4.9	43.6	16.7	4.8	39.5	16.0	4.0	Recovered
11	3	41.5	11.9	4.9	38.8	14.9	5.4	51.6	22.0	4.8	50.0	21.0	4.4	Recovered
12	4	38.0	15.7	4.7	36.3	13.9	5.0	34.9	12.7	5.5	34.0	12.6	5.2	Recovered
13	5	31.6	13.8	4.8	36.1	16.2	6.0	40.2	16.4	6.0	39.1	16.2	5.4	Recovered
14	5	39.6	17.3	6.3	37.5	15.0	6.5	38.0	15.2	6.2	37.8	15.0	5.7	Recovered
15	5	44.0	20.0	6.2	47.0	16.7	5.8	49.8	19.0	6.0	49.2	18.7	6.0	Recovered
16*	5	37.2	12.4	6.2	34.2	13.6	5.7	42.0	18.7	6.0				Tapes replaced
17*	5	38.8	15.2	5.6	37.8	15.5	5.8	47.0	19.0	7.2				Tapes replaced
16	8				31.4	15.6	5.7	35.1	17.7	6.6	34.2	15.5	5.7	Recovered
17	8				38.3	18.3	5.4	38.5	18.2	5.2	30.2	15.1	4.5	Recovered
Ave...19		39.8	15.1	5.8	45.0	18.4	6.2	51.6	21.2	6.5	43.2	18.4	4.8	

* Bandaged legs placed in ice bath for 48 hours (see text).

Bacterial infection, observed frequently in the first non bandaged control animals, developed within 24 to 36 hours in the bandaged legs. Extensive accumulations of gas appeared in the regions of the thighs and lower abdomen, with the animal rarely surviving more than 24 hours after their first appearance. Smears made of the serous exudate from the gas filled tissues of a badly infected dog showed at least four different groups of micro-organisms. Further cultural investigation under aerobic and anaerobic conditions revealed the presence of *a*, Clostridium welchii; *b*, Clostridium sporogenes; *c*, Streptococcus putridus, and

d B. coli.[3] Wilson and Roome (5) reported the presence of an unidentified anaerobic gas forming bacillus in the constricted legs of 8 of 11 dogs. They recovered the same or a closely similar organism from the normal legs of 7 of 11 animals.

To reduce the incidence of these infections, the limbs of the dogs were closely clipped with an electric clipper and the skin of the legs and toes thoroughly washed with green soap. A solution of picric acid was then applied, followed by 80 per cent alcohol. After the constrictions were placed, the legs were lightly covered with towels which were kept soaked with alcohol. Bandages placed on the legs after release of the tourniquets were moistened with alcohol and kept wet as long as they were on the limbs. This procedure, while tedious, did successfully prevent infection, showing that the probable source of the organisms producing the infection was the skin of the devitalized limbs.

Allen (6) has stressed the point that lowering the temperature of the ligated limb reduces the danger of shock and subsequent gangrene, and enables the affected part to tolerate lack of circulation for a longer time. The ameliorating effect of chilling has been attributed to a depression of tissue metabolism and reduced formation of toxic substances in the ligated extremities. Our experience with reduced temperature has been confined to packing the bandaged limbs in ice after release of the constrictions, merely as a means of retarding bacterial growth. In this regard the use of ice was successful, but the procedure was so cumbersome that it had no advantage over keeping the bandages wet with alcohol. Furthermore, the ice treatment seemed definitely to retard the rehabilitation of the vessels of the injured legs, so that a longer interval was required before the wrappings could safely be removed (table 3).

Effect of saline injections and plasma transfusions. Wilson and Roome (5) reported that transfusions of large amounts of citrated plasma and saline were ineffective. The administered fluid failed to remain in the general circulation but was lost into the tissues of the damaged limbs. Allen (6) states that early and abundant injections of saline allowed recovery when injections of blood or plasma failed to save life.

In our hands saline infusions, even in large quantity, were without effect (table 2). It did not matter whether the salt was given immediately following release of the constrictions, or later. The hemoconcentration was not relieved and the legs showed massive edema. The rate of leg swelling seemed directly correlated with the amount of saline administered and the rate at which it was given.

Plasma transfusions. These were of two types: 1, continuous, and 2, intermittent. The efficacy of the transfusion was found to depend upon the manner in which it was given. In both types the total quantity of plasma was the same, as calculated on a body weight basis.

A. *Continuous transfusion given immediately following release of the tourniquets.* The most logical time for the administration of a transfusion would seem to be

[3] We are indebted to Mr. George Warren of the Section of Microbiology for identifying these organisms.

immediately following the release of the constrictions, or just before the animal entered the period of shock. Transfusions were therefore begun within 15 minutes of the release of the constrictions and continued over a period of 30 to 50 minutes. This treatment failed to relieve the hemoconcentration (fig. 2). Nor did the transfusion serve to prevent or even postpone death (table 2). The fact that the legs continued to swell, that the hemoconcentration became progressively more severe, and that the serum protein concentration declined steadily from the high level reached immediately following the transfusion, makes it evident that the administered plasma failed to remain in the circulation but leaked into the leg tissues.

Of 6 animals given plasma transfusions in this manner, only 1 survived (table 2). This dog failed to exhibit a typical degree of hemoconcentration even in the pre-transfusion period.

B. *Continuous transfusion given three hours after release of the tourniquets.* Untreated control animals showed the greatest part of the total plasma loss immediately after removal of the constrictions. A transfusion given during this period of rapid leakage not only did not reduce this loss, but the plasma administered was also lost from the blood stream. It is conceivable, therefore, that the injected fluid, by increasing the fluid transfer through the injured capillary bed, was actually detrimental to the animal. On the other hand, a transfusion given after this initial plasma volume depletion, even though the animal was now in shock, might prove more efficacious. To check this possibility, continuous transfusions were given a second series of animals after an interval of three hours had elapsed since removal of the constrictions. At this time the maximum changes in hemoconcentration had occurred (fig. 2) and early shock symptoms were present.

The response to such delayed transfusions was better than that to the transfusion given immediately upon release of the constrictions, but the number of animals which recovered was small (table 2).

C. *Intermittent transfusions of small amounts of plasma.* If instead of giving single transfusions over 30 minutes, the total amount of plasma was divided into five equal portions and transfused at the time of release of the constrictions, and at the end of the first, second, fourth, and sixth hours thereafter, the effect in preventing shock was truly dramatic. The animals all recovered and ate within a few hours of recovery from the anesthesia. Unlike the bandaged animals, they showed no gangrene after several days. The legs were paralyzed, but otherwise the animals remained in perfect health and could be kept as long as desired.

The clue to the dramatic response to the intermittent transfusion is not afforded by a survey of the hemoconcentration and plasma volume changes (fig. 2). The data indicate that small intermittent transfusions are not retained in the blood stream any more readily than is the continuous transfusion. Nor is the absolute level of hemoconcentration different from that of either series of animals receiving the continuous transfusion.

The critical factor allowing survival is not merely the ability to dilute the

blood or lower the hemoconcentration, for dilution progresses but slowly in the animals of all series. Complete restoration to the normal hematocrit levels in intermittently transfused animals, is rarely complete by the 48th hour, even though the animal is in comparatively good health. The evidence is suggestive therefore that the striking beneficial effects of small amounts of plasma given intermittently cannot be fully accounted for by a mere physical dilution of the circulating blood volume.

Discussion. The rapid hemoconcentration, coinciding with marked swelling of the legs, indicated that undue transudation of plasma through the injured capillaries into the legs, with a consequent sharp reduction in circulating blood volume, is the initiating factor in the shock which follows release of the constrictions. The fact that merely tightly bandaging the limbs immediately after release of the constrictions will prevent the circulatory failure, seems strong evidence for this conclusion. It is not to be inferred, however, that death is due solely to the extreme hemoconcentration and reduction in circulating blood volume, for hematocrit levels fully as high may be reached in experimental animals (e.g., with intraperitoneal glucose injections) without leading to a fatal issue. Also, the restorative action of the intermittent transfusion cannot be correlated with hemodilution changes.

It has been assumed by some that the actual shock following release of constrictions on the extremities is due to a flooding of the systemic circulation with toxic products from the injured area. The present experiments present some evidence against this view: 1. The circulation of the bandaged limb is patent at all times, as shown by the recovery of dye injected into the leg veins. Any toxin present in the legs should therefore have free access to the general circulation. Yet bandaged animals exhibit no signs of shock. 2. Removal of the bandages within the first 24 hours is followed by swelling of the legs, hemoconcentration and death. Replacement of the bandages, if done promptly, before the hemoconcentration has become well advanced, will promptly stop further leg swelling and hemoconcentration and allow normal recovery of the animal. The longer the interval allowed before the bandages are removed, the less the subsequent hemoconcentration and the more rapid the recovery of the animal from the temporary concentration. Apparently the minute vessels of the limbs, if given sufficient time, gradually recover to the point where excessive plasma leakage does not occur. The ability of the intermittent transfusion to prevent shock in an animal without bandages or other means of preventing local fluid loss, without inducing an appreciable hemodilution, is not clear. Further experiments are in progress to attempt a more complete explanation of this point.

SUMMARY

1. The tourniquet method for producing shock, in which both hind legs were constricted by heavy walled rubber tubing tightly tied around the hips for a period of 5 hours, has been studied in a large series of dogs.

2. Of 25 untreated control animals, all but one died in shock following the release of the constrictions. The survival periods ranged from 3 to 27 hours.

Associated with the shock condition was a marked swelling of the injured legs, an intense hemoconcentration, and a plasma volume reduction of 49 per cent.

3. In all animals in which leg infections were prevented by proper antiseptic treatment, the snug bandaging of both legs prior to, or immediately after removal of the constrictions, successfully prevented shock. While shock was prevented by the bandaging, the legs were paralyzed and recovered but slowly.

4. A plasma transfusion of 25 cc. per kgm. body weight, given immediately after release of the constrictions or later, failed to prevent the hemoconcentration, and did not prevent death in 9 of 12 dogs.

5. The same amount of plasma, divided into five doses of 5 cc. per kgm. body weight each, and transfused intermittently over a seven hour period, prevented shock in all of 7 dogs. This positive effect could not be correlated with hemodilution changes.

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CARDIAC INJURY POTENTIALS[1]

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A localized region of injury of cardiac muscle, electrically negative when the heart is at rest, becomes electrically positive, with respect to the potential of resting uninjured muscle, when the muscle contiguous to the injury enters into activity (1) (2) (3). The present report is concerned with certain characteristics of this phenomenon, particularly its development when injury is produced and its relation to the contraction of heart muscle.

METHODS. For the production of localized injury of surface regions on the heart and the recording of the resulting injury potentials, a suction electrode of the type first described by H. C. Wiggers (4) was employed in all experiments. A small tab of heart muscle is sucked into a glass tube of 1 mm. internal diameter and makes contact with the wick of a zinc-sulphate electrode. In most cases the other electrode completing the circuit to the amplifier input was placed in contact with a hind leg of the animal (unipolar recording). The amplifiers used in connection with the cathode ray oscillographs are balanced, two channel, direct coupled and entirely free from detectable amplifier drift at amplifications considerably higher than those used in the experiments. Figure 1A shows the response to a potential change in the input circuit of 1 mv.

In the experiments concerned with intraventricular pressure, a membrane manometer provided with a photo-electric cell was used (5). All measurements from the curves were made with a micrometer comparator. Most of the experiments were carried out on the exposed hearts of large specimens of the snapping turtle (Chelydra Serpentina) after destruction of the brain and spinal cord and on the exposed hearts of dogs under ether or nembutal anesthesia.

Characteristics and development of the injury potential. The injury potential-time curves from the heart recorded by the use of the suction electrode are remarkably consistent in their contour in the same and even in widely different animal species. Figure 1 is a record resulting from suction applied to the exposed heart of Limulus. In this, as in all records, unless noted otherwise, the recording is unipolar. Downstroke in all records is in the direction of positive potential at the heart contact. The principal differences from the injury potential of Limulus and similar potentials recorded from the heart of the carp (fig. 2) and from the hearts of turtles and dogs (figs. 3 to 7) are in the more rapid decline in potential following the positive maximum and in the presence of superposed oscillations on the declining limb.

One of the most interesting features of the injury potential is its localized character. When a unipolar lead is brought along the surface of the heart successively nearer to a region of injury, no trace of the injury potential is evident

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until the contact is within about a millimeter from the margin of the injury. Another indication of this localization is the fact that the curve of an extrasystole differs not at all or only in minor ways from the curve of a normal heart cycle. Figure 3 is a record of an extrasystole in the turtle's ventricle produced by a condensor discharge shock applied at some distance from the suction electrode. In the extrasystolic cycle (third cycle) the time rate of change in voltage in passing from the state of maximum negativity to the state of maximum positivity is somewhat less than in the normal cycle. Figure 4 is a similar record from a dog's ventricle. In this the maximum rate of voltage change, in contrast to the turtle record, is slightly greater in the extrasystolic cycle than in the normal cycle. The localized character of the injury potential is no doubt dependent on the nature of the potential distribution in the form of concentric rings of positive and negative charges (1) (2). If the seat of injury is pictured as a localized cup shaped depression in the muscle with this kind of charge distribution, there is to be expected no resulting potential difference on the heart surface. On the other hand, if the heart containing an injured region is surrounded by an electrically conducting field, as it is in the intact animal, a potential field would be established in which potentials would be apparent at all points at which the solid angle subtended by the injury is not zero and of a magnitude proportional to this angle. We have proven this to be true experimentally by immersing an isolated heart containing an injured region in a saline bath and plotting the potential field around it. It would seem clear that it is the potential field resulting from injury potentials in an ischemic region of heart muscle which is responsible for the characteristic electrocardiographic changes which occur in this condition. An ischemic region of the dog's ventricle develops injury potentials which resemble those produced by other forms of injury.

It should also be clearly recognized that with the heart in situ, any experimental procedure which produces injury to the heart surface will cause modifications in electrocardiograms which result directly from the injury potentials developing in the region so injured. Efforts to determine the contribution to the normal electrocardiogram of particular regions of the heart by attempting to prevent normal action potential development from other regions by injuring the latter (6), merely results in the replacement of the normal action potentials by injury potentials. Since it is obviously impossible to ablate any region without producing injury, and since injury of any type produces large and sustained injury activity potentials which directly modify the electrocardiogram, no conclusions can be drawn as to the normal distribution of potentials from experimental procedures of this type.

Injury potentials develop fully within one or two cycles following the application of suction to a surface region of the turtle heart. If the suction is started during systole, the initial potential change is in the positive direction and to an amount which is approximately the same as that which occurs at the same point in the cardiac cycle in subsequent cycles. In figure 5 two examples are given of the development of injury potentials resulting from the application of suction

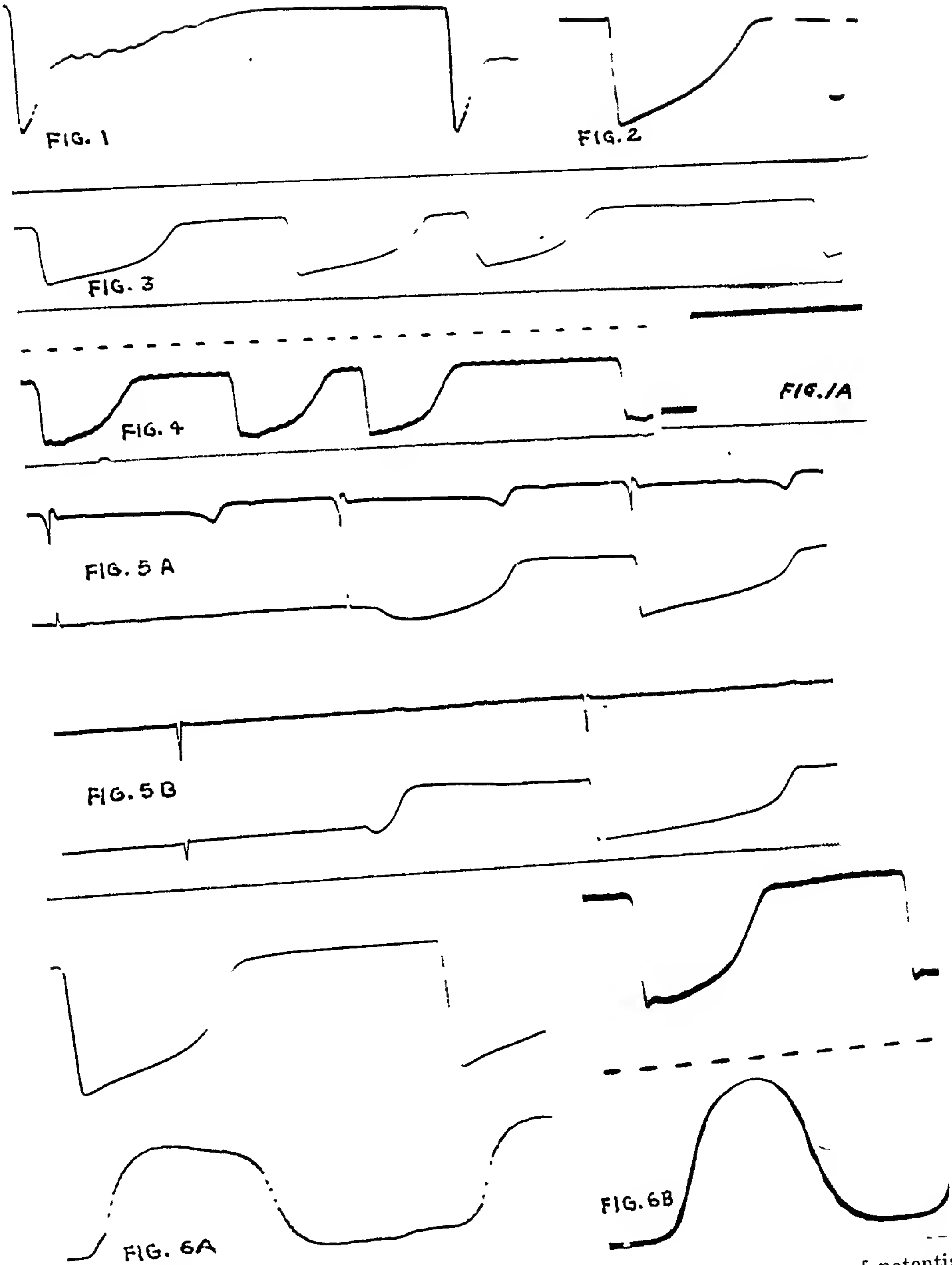

Fig. 1A. Response of one of the amplifier-oscillograph units to a change of potential in the input circuit of 1 mv.

Fig. 1. An injury potential-time curve recorded from the exposed heart of Limulus.

Fig 2. A similar curve as in the preceding figure from the exposed heart of a carp. The

to local regions of the turtle's ventricle. The upper records in each case are reference curves, the lower curves are unipolar leads from the suction electrode. In A, suction was started early in systole in the second cycle shown. A positive potential developed which reached a maximum value of 11.1 mv. Before the end of systole, as indicated by the T wave on the reference curve, the positive potential began to decline and the region later became the seat of a negative potential which reached a maximum value of 20.8 mv. during the diastolic period. With the onset of the following systole, the potential sign reversed rapidly to reach a positive potential maximum of 18.6 mv. In B, the suction was started considerably later in the systolic period. The first change in potential was again in the positive direction, but the maximum positive potential reached in this cycle was only 4.0 mv. The potential then reversed in sign to reach a maximum negative potential of 24.4 mv. during diastole, followed by a maximum positive potential of 17.0 mv. early in the next cycle.

In the dog, injury potentials develop more slowly following the application of suction and may not be completely developed until after some ten or fifteen cycles.

The time relations of ventricle injury potentials and intraventricular pressure. Simultaneous recordings of injury potentials from various regions on the surface of the ventricle along with intraventricular pressure, show that the injury activity potential starts before the rise of pressure begins. The maximum positive potential falls within the initial period of slow rise of intraventricular pressure.

In figure 6 there are reproduced records of injury activity potentials and intraventricular pressure in the turtle and in the dog. In the turtle record (A), the injury activity potential starts 0.01 sec. before the start of the rise of pressure. The maximum positive potential and the end of the initial slow rise of pressure are coincident. The potential returns to the diastolic level 0.251 sec. before the end of the fall of intraventricular pressure. In the dog record (B)

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calibration curve at the right in this figure records a difference of potential across the input of the amplifier of 20 mv.

Fig. 3. Comparison of the injury potential-time curve in an extrasystolic cycle with normal cycles from the ventricle of a turtle. The first two cycles shown are normal and the third cycle is the extrasystole, followed by a compensatory pause. The extrasystole was caused by a brief condensor discharge applied to the base of the ventricle about 2.5 cm. distance from the injury. For further discussion see text. Speed of recording, 15 mm./sec.

Fig. 4. Similar record to that of figure 3 made from the anterior surface of the right ventricle of a dog. The extrasystole is the third cycle shown. For further discussion see text. Speed of recording, 69 mm./sec.

Fig. 5. The development of injury potential from the application of suction at different times in the cardiac cycle of the turtle ventricle. The upper curves in A and B are differential curves from the base of the ventricle and are used as reference curves. In A, suction was applied early, in B late in the systolic period. For further discussion see text. Speed of recording, 15 mm./sec.

Fig. 6. The upper curves are injury potential-time curves from a ventricle region, the lower curves intraventricular pressure. A is from a turtle, B from a dog. A rise of the lower curve indicates a rise of pressure. For further discussion see text. A was recorded at a speed of 20 mm./sec., B at a speed of 69 mm./sec.

the injury activity potential starts 0.008 sec. before the start of the pressure rise. The positive potential maximum occurs 0.014 sec. before the end of the initial

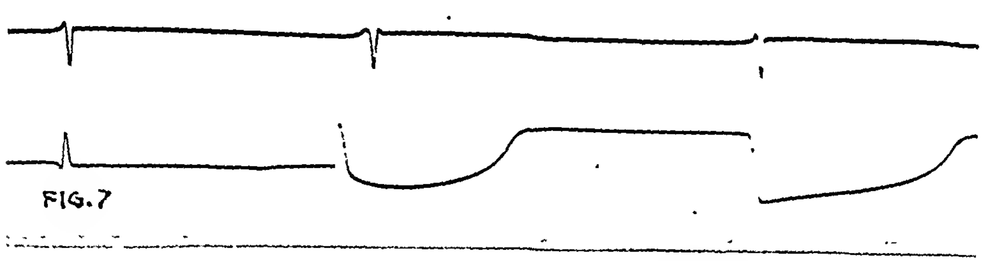

FIG. 7

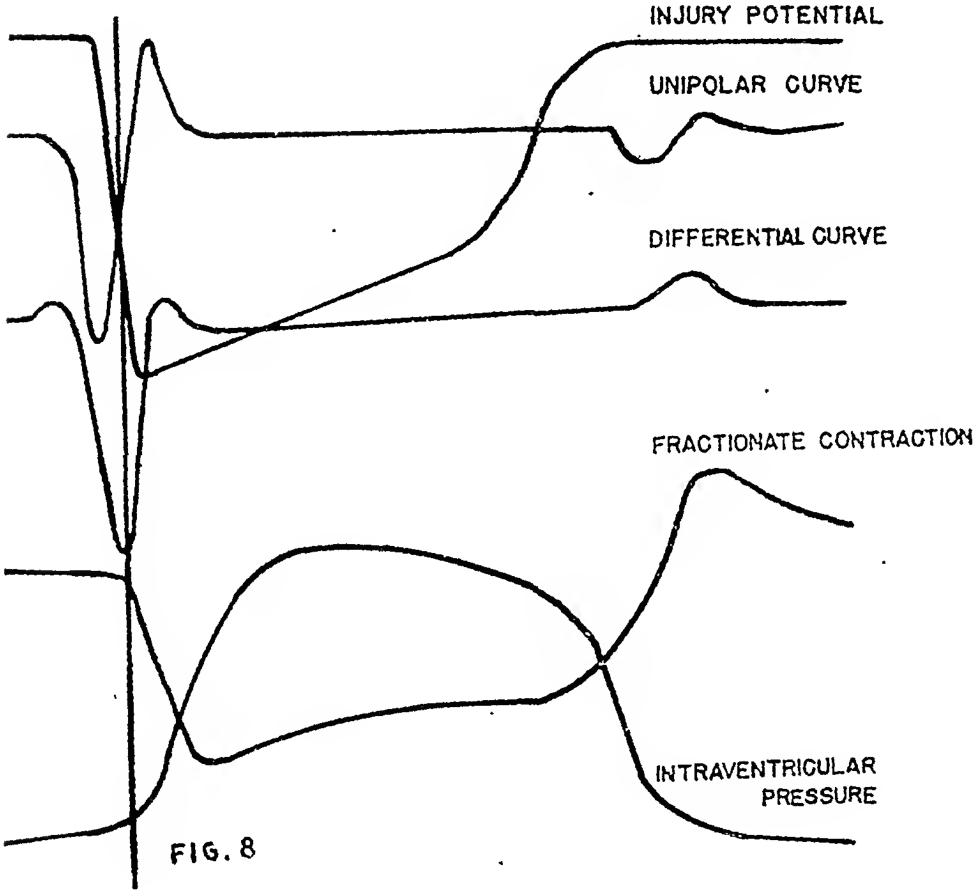

FIG. 8

Fig. 7. Illustrates the type of record from which the onset of fractionate contraction in a region before injury is produced, is compared with the onset of the injury potential-time curve from the same region after injury is produced. The upper curve is a differential curve from the ventricular base and serves as a constant reference curve. The first cycle of the lower curve is a differential potential-time curve from a region near the midventricle to which suction was applied and injury produced near the end of this cycle. This record was made from a turtle. For further discussion see text. Speed of recording, 15 mm./sec.

Fig. 8. Comparison of the time relations of certain electrical and mechanical activities of the heart. For discussion, see text.

slow rise of pressure and the potential returns to diastolic level 0.099 sec. before the end of the fall of intraventricular pressure.

The relation of the injury activity potential to the onset of fractionate contraction of surface regions of the ventricle. While it is most probable that an injured region of heart muscle does not contract, the muscle at the margin of the injury does, and the contraction, or some process associated with the contraction of the muscle immediately surrounding the region of injury is responsible for the production of the injury activity potential. It is therefore of especial interest to obtain, if possible, the time relations of the injury potential and the contraction of the muscle contiguous to the region of injury.

It has been recently shown (7) (8) that the onset of fractionate contraction at surface regions of the ventricles of the turtle and dog is coincident, within small errors of measurement, with the occurrence of the main peak of the differential potential-time curve recorded from the region. In an effort to relate the injury activity potential to contraction of the contiguous muscle, we have recorded differential curves from regions before injury and injury activity potentials from the same regions after suction injury was produced. For these experiments a suction electrode was altered to serve also as a differential electrode by mounting a wick electrode on the outside of the suction tube so that contact with the heart surface is made at the outside margin of the tube. When this electrode is applied to a surface region without suction, it represents two electrode contacts approximately a millimeter apart, sufficiently close to meet the requirements for a differential lead. After recording the differential curve along with a constant reference curve suction is applied and the injury activity potential recorded from the same region. The procedure is illustrated by figure 7 from an experiment on a turtle. The upper curve is from a differential lead and serves as a constant reference curve. The first cycle of the lower curve is a differential curve recorded by means of a suction electrode modified as described. Toward the end of this cycle suction was started. The lower curve moves in the direction of negative potential and then quickly reverses to a positive potential in the next systole. In the third cycle shown, the injury activity potential is fully developed. Measurements of these curves show that in the normal cycle preceding suction, the main differential peak precedes the peak of the reference curve by 0.0416 sec. In the cycle after the injury activity potential had developed, the onset of the potential change precedes the reference peak by 0.1260 sec. By taking a point 0.0416 sec. before the reference peak, this point falls approximately midway between the onset of the injury activity potential and its maximum when the voltage change in the positive direction has reached 20.2 mv. The total voltage change from the negative potential of rest to the maximum positive potential is 44.9 mv.

We have made measurements of this type from 69 records obtained from 8 turtle hearts and from 29 records from 2 dog hearts. In all cases it was found that the main differential peak, recorded before injury was produced, coincided in time with the period on the injury potential curve, recorded after injury, during which the potential is changing rapidly in the positive direction. From these results it is concluded that the injury activity potential starts in a region before fractionate contraction begins in the muscle contiguous to the injury

and that the onset of contraction in the surrounding muscle occurs at a time when the potential of the injured region is changing most rapidly from the electronegative state characteristic of rest to the electropositive state characteristic of activity. That the injury activity potential starts before contraction is also evident from the fact that its onset from all surface regions precedes the rise of intraventricular pressure, as noted in the preceding section.

Discussion. The time relations of electrical and mechanical events in the heart, as derived from the results of the present and preceding experiments (7) (8) are shown diagrammatically in figure 8. The curves shown were all redrawn from original records in which two of the curves were recorded simultaneously. The onset of fractionate contraction at the various surface regions of the ventricle occurs during the initial period of slow rise of intraventricular pressure. At any one region, the onset of contraction is coincident with the main peak of the differential curve and with the maximum time rate of change of the unipolar curve. The onset of the injury activity potential precedes these events by a definite interval.

The injury activity potential differs from other electrical potentials that may be obtained from the heart in that it *starts* at different time instants at different surface regions and that the start is definitely related to the onset of fractionate contraction in the particular region. The start of the injury activity potential precedes the onset of fractionate contraction at every region by an interval which is nearly constant, especially in the same heart. It would seem obvious that some changes are occurring in the muscle contiguous to the injured region which precede the contraction of the muscle and which are associated with a rapid change of potential of the region. The view that some such change occurs preceding contraction, commonly ascribed to the depolarization of a polarized membrane, has long been prevalent. This hypothetical process has been generally known as "the process of excitation" or "impulse" and has been identified with the occurrence of a local fall of electrical potential or "negativity." On the other hand, the two types of potential-time curves which may be employed to reveal the electrical state of the normal muscle, the unipolar and differential curves, clearly disprove the "negativity" hypothesis but show no feature which can be identified with the start of the excitation process.

In a preceding paper (1) it was postulated on theoretical grounds that the injury potentials are associated with concentric rings of positive and negative charges at the margin of the injury, which reverse in polarity when the surrounding muscle enters into activity. Because of the presence of an apparently new disposition of electrical charges and because of the large and prolonged potential change, which lasts throughout the whole period of contraction, we expressed the opinion that the injury potentials are the expression of new charge distributions that do not exist in uninjured regions. Sugarman, Katz and Jochim (2) constructed a model consisting of concentric metal rings placed in a saline bath and connected to a source of alternating current. They found that the potential distribution shown by this model was similar to that in a cardiac muscle injury. From these experiments the conclusion was drawn that the

injury potentials of the heart could be explained in terms of the theory of the polarized membrane, if one assumed that the injured region was in a constant state of partial depolarization. The alternation of the surrounding normal muscle from a state of maximum polarization to complete depolarization would account for the resting and activity injury potentials without the assumption of any new source of potential. Although this ingenious explanation is of interest it should be pointed out that with our present lack of knowledge as to many of the details of the electrical state in heart muscle associated with injury, particularly as regards electrical resistance relations, any models composed in part of low resistance metal conductors may be far from a true representation of the electrical state present in the heart. The observations quoted previously in the present paper regarding the electrical field established by a heart injury when immersed in a volume conductor would indicate that a model formed of concentric segments of two spheres of unequal radii would be perhaps a nearer approximation. Any conclusions drawn from results obtained by models supposed to represent the electrical conditions present in and around a cardiac injury, must, with our present meager knowledge, be considered with great reserve. The best model that we have of the distribution of injury potentials is the living preparation itself.

The marked constancy of the injury activity potential in form and magnitude from different regions of the heart and the fact that it differs to such a marked degree from normal action potentials recorded from the same regions before injury, makes it difficult to assume that it is entirely a representation of a process that occurs in uninjured muscle. The unipolar curves from different regions have characteristics which depend on the particular region, the potential of which may be initially positive or negative and undergo two or three reversals. The character of this curve is usually profoundly modified in an extrasystole produced by artificial stimulation. The contour and magnitude of the injury activity potential are essentially the same from all regions in normal beats and in extrasystoles. It seems most probable that it may be considered, in large part at least, as independent of the potential changes which occur under normal conditions in the surrounding muscle. It may well be, however, that the initial part of this potential change, the start of which precedes contraction by an approximately constant interval, signals some phenomenon which normally occurs in contiguous tissue before the mechanical process of contraction starts.

SUMMARY

The potential time curve, derived from an injured region of heart muscle by the use of the suction electrode, is remarkably similar in contour and magnitude when recorded from different regions of the same heart, from different animals of the same species and even from animals widely separated in the animal scale. It represents a local potential change which does not involve the normal muscle contiguous to the injury, although a potential field develops in a conducting field which surrounds it on all sides. The contour and magnitude of the curves differ little in extrasystoles as compared with normal beats, in con-

trast to the marked differences in unipolar and differential potential time curves in these two circumstances.

The change in an injured region from a negative potential to a positive potential, which occurs when the muscle contiguous to the injury enters into activity, is due to some process in the contiguous uninjured muscle or at the boundary between the injured and uninjured muscle. The start of this potential change at all surface regions of the ventricle precedes the rise of intraventricular pressure by an interval which varies with the location of the injury. At each local region, its onset precedes the onset of local or fractionate contraction of the region by an approximately constant interval.

The possible relation of the start of the injury activity potential to the "excitation process" or "impulse" in the contiguous active muscle is discussed.

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THE INFLUENCE OF GROWTH ON THE PHOSPHORUS METABOLISM OF THE MOUSE AND THE EFFECT OF THYROXINE AT VARIOUS AGES

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Although it has been known for some time that the phosphorus turnover of various tissues decreases with age, probably corresponding to the fall in basal metabolic rate with age, little specific data are available on this point. In this connection we have compared the phosphorus turnover of several tissues of the mouse at three age levels, namely, 6, 12 and 24 weeks.

Since thyroxine is known to cause a general increase in metabolism, it was of interest to determine the effect of the hormone on phosphorus metabolism. For this purpose we have compared the total phosphorus turnover of the various tissues of thyroxine-treated mice of these age levels with that of control animals.

METHODS. In this investigation, young albino mice of three age groups (6, 12 and 24 weeks) were used, having average weights of 11.5, 22.0 and 27.0 grams, respectively. They had been maintained on a diet of oats and Purina dog chow.

In each group the experimental animals received intraperitoneally on two successive days a dose of 0.05 mgm. of crystalline thyroxine (Roche-Organon) dissolved in 0.2 cc. of 0.01 N NaOH, while the control animals received 0.2 cc. of 0.01 N NaOH. All animals received intraperitoneally radioactive phosphorus in the form of Na_2HPO_4 24 hours after the last thyroxine administration and were sacrificed by decapitation 24 hours after the injection of the labeled phosphorus.

The liver, brain, spleen, kidney, heart and tibia were removed for analysis; the blood was collected at decapitation. The tissues for study were weighed as soon as possible after removal from the body and then dissolved in fuming nitric acid. Their radioactivity was determined on a scale-of-four Geiger Müller counter (1). In addition, the total phosphorus content of the various tissues was determined by the colorimetric method of Fiske and Subbarow (2). Our results are expressed as the specific activity of the tissues per gram mouse (percentage of the original dose of P* per milligram P in 1 gram of tissue per gram mouse).

RESULTS AND DISCUSSION. Our data are treated in three sections: 1, distribution of labeled phosphorus in the mouse; 2, the effect of age on the phosphorus turnover, and 3, the effect of thyroxine on the phosphorus turnover of the mouse.

Distribution. The distribution of labeled phosphorus in the mouse is very similar to that found in the rat and other species (3). Expressing our values as per cent dose per gram tissue, we find that tibia shows the highest turnover of

phosphorus followed in descending order by spleen, kidney, liver, heart, blood and brain. A similar order of tissues is reported by Lawrence (4) and associates and by Hodge et al. (unpublished data) on the distribution of phosphorus in the various tissues of the normal mouse. Likewise Cohn and Greenberg (5) in a study of the phosphorus distribution in the young adult rat found that bone shows the largest retention (in per cent dose per gram), followed by liver, stomach and small intestine, heart, kidney, blood and brain.

Effect of age. By choosing growing mice we were able to show the changes in the rate of phosphorus turnover in mice with age increase. In table 1 are shown the specific activities of the various tissues of the three age groups under consideration. These values show a statistically significant decrease in the rate

TABLE 1

Average values for phosphorus turnover in various tissues of albino mice, ages 6, 12 and 24 weeks

	PERCENTAGE DOSE RADIOACTIVE PHOSPHORUS PER MILLIGRAM PHOSPHORUS‡						
	Blood	Liver	Brain	Spleen	Kidney	Heart	Tibia
			Group I. 6 weeks old				
Controls		3.10 (10)†	0.34 (10)	2.18 (10)	1.94 (9)	1.41 (8)	0.94 (8)
Experimentals		2.78 (10)	0.32 (10)	2.46 (10)	2.19 (10)	1.40 (10)	0.82 (7)
Entire group		2.94 ±1.17*	0.33 ±0.11*	2.32 ±0.58*	2.07 ±0.78*	1.40 ±0.47*	0.88 ±0.26*
			Group II. 12 weeks old				
Controls	1.55 (5)	2.48 (5)	0.16 (5)	1.44 (5)	1.58 (5)	1.31 (5)	0.21 (5)
Experimentals	1.51 (4)	2.35 (4)	0.19 (4)	1.59 (3)	1.47 (4)	1.08 (4)	0.28 (4)
Entire group	1.53 ±0.17*	2.42 ±0.48*	0.17 ±0.04*	1.49 ±0.11*	1.53 ±0.23*	1.21 ±0.15*	0.24 ±0.04*
			Group III. 24 weeks old				
Controls	1.25 (5)	1.56 (5)	0.11 (5)	0.96 (5)	1.05 (5)	0.82 (5)	0.08 (5)
Experimentals	1.09 (9)	1.46 (10)	0.12 (10)	0.86 (9)	1.14 (10)	1.08 (10)	0.13 (10)
Entire group	1.15 ±0.23*	1.49 ±0.29*	0.12 ±0.03*	0.90 ±0.28*	1.11 ±0.23*	0.99 ±0.32*	0.11 ±0.03*

* Average deviations.
† Figures in parentheses are numbers of mice used.
‡ Expressed per gram mouse in each case.

of phosphorus turnover of each tissue with increasing age.[1] With an age increase from 6 to 24 weeks, there is a decrease in the phosphorus turnover in the liver of 61 per cent, in the kidney of 46 per cent, in the heart of 29 per cent, and in the tibia of 87 per cent (table 1). These observations are in line with those of Weissberger and Harris (7) who found a similar decrease in phosphorus turnover of blood, kidney, genitals and femur with increasing age of the rat. In table 1 data on the phosphorus turnover of the brain show a decrease of 64 per cent in the period observed. This decrease in total phosphorus turnover is similar to that reported by Chaikoff (8) and his collaborators who studied *in vivo* the phospholipid phosphorus turnover of the central nervous system of the rat at various ages and found that the rate of the incorporation of P* into phospholipid fell off

[1] Statistical procedure according to R. A. Fisher (6), p. 128.

markedly with age. These findings were further substantiated by the recent *in vitro* studies by the same author (9), on the manufacture of phospholipid by the brain. In general, the results in regard to the decrease in phosphorus turnover of various tissues with time are consistent with the histochemical findings of Lowry (10) and his associates who observed that the concentration of phosphorus in the cardiac muscle of the rat diminishes with growth.

Figure 1 shows the slope of the decrease in phosphorus turnover with age for each of the tissues studied. The rate of turnover is clearly seen to be a function of the age of the animal. However, there is no direct correlation between the specific activity of a given tissue and the percentage fall in its specific activity with age, i.e., the tissues with the highest specific activity do not necessarily show the greatest decrease with age. Thus, the descending order of the tissues according to their specific activities at 6 weeks is liver, spleen, kidney, blood, heart, tibia, and brain. However, this order, according to the percentage decrease in the specific activities from 6 to 24 weeks, is tibia, brain, liver, spleen, kidney, blood and heart.

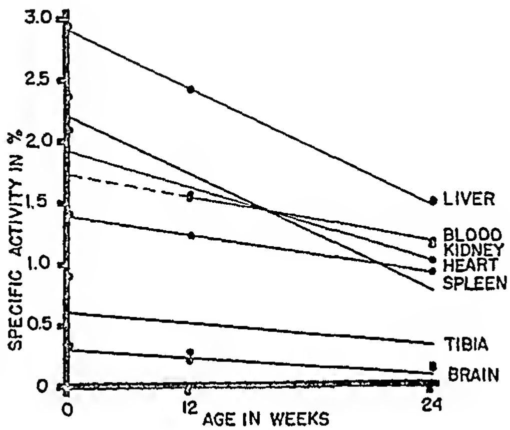

Fig. 1. The decrease in phosphorus turnover with age. At the age of 6 weeks the liver of the mouse has the most active phosphorus turnover; next are the spleen, kidney, blood, and heart in a group, and much lower are the tibia and brain. In the period from 6 to 24 weeks all the tissues show marked decreases in their respective rates of phosphorus turnover. The liver and spleen have the largest decreases in specific activities, the tibia has the greatest percentage decrease (87 per cent), and the brain shows the least change. At 24 weeks the tissues in descending order of activity are liver highest, then blood, kidney, heart and spleen in an intermediate group, and brain and tibia least.

During early growth, large amounts of phospholipid are being laid down in the brain, large amounts of inorganic phosphorus in the bone; phosphorus compounds are not being incorporated into the viscera to as great an extent. Consequently, it is interesting to compare the decrease in phosphorus turnover of these tissues for the two age periods under consideration (6 to 12 wks. and 12 to 24 wks.). This decrease is significantly larger for brain and tibia during the first period than during the second, whereas the specific activities of the viscera decline to about an equal extent for each period.

Effect of thyroxine. Since thyroxine is known to have an effect on the basal

metabolic rate, an increase in the phosphorus metabolism was also expected. However, our findings have led to no such general conclusion. The difference between the thyroxine-treated animals and the control values is not statistically significant in any of the tissues investigated, with one exception; there is a valid increase in the phosphorus turnover of the thyroxine-treated older animals (groups II and III, aged 12 and 24 wks. respectively) in the tibia only. There is no such difference, however, in the youngest group of animals (table 1). This may be due to the fact that the phosphorus turnover is extremely high in the young animal and, therefore, the relatively small change induced by the administration of thyroxine may not be demonstrable under our experimental conditions. Belasco and Murlin (11) have reported that in very young rats thyroxine shows less effect on the basal metabolic rate than it does in older animals. In the other tissues (table 1) the apparent slight tendency toward a decrease in turnover following thyroxine administration can be ascribed in all probability to individual variation and experimental error. Manly, Hodge, and Manly (12) have shown that about one-sixth of the bone (so-called labile bone) is in a high metabolic state at all times. From this point of view it is not surprising that the bone phosphorus metabolism is particularly sensitive to thyroxine.

This increase in phosphorus turnover of the tibia following thyroxine treatment has an interesting bearing on the observation of Karnofsky and Cronkite (13) who showed that an excess of thyroid hormone in the rat will produce more rapid appearance and unification of the ossification centers in the bones and a more rapid eruption of the teeth. Furthermore, Silverberg and Silverberg (14) found that in growing mice the administration of thyroxine accelerates and intensifies the age changes of the skeleton. This intensification and acceleration of the skeletal changes which accompany growth is in agreement with our findings of the increased phosphorus turnover of the tibia of the growing thyroxine-treated mouse.

SUMMARY

In the growing mouse the rate of phosphorus turnover of the tissues studied is a function of the age of the animal.

The decrease in the rate of phosphorus turnover with age is greatest in tibia, followed in descending order by brain, liver, spleen, kidney, blood and heart.

Thyroxine seems to have very little, if any, effect on the phosphorus turnover of the tissues of the mouse, with the exception of the tibia which in the older mice shows an increase in the P* to P ratio upon the administration of thyroxine.

The author wishes to express her appreciation to Dr. H. C. Hodge and Dr. L. H. Weissberger for their interest and help; to Dr. G. Dessauer for the preparation of the radioactive phosphorus, and to Dr. W. F. Bale and Mr. John Bonner for the maintenance of the Geiger-Müller counters.

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LITTER SIZE, GROWTH RATE AND HEAT PRODUCTION OF SUCKLING RATS

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The growth rates of suckling mice (1) and pigs (2) depend on litter size. The smaller the litter the greater the relative milk supply and the more rapid the growth rate. This paper reports data on the influenee of litter size on the growth rate of rats during the suekling period (birth to 21 days) and especially on the relative heat productions of the rapidly and slowly growing suckling rats.

Tangl (3), Farkas (4), Bohr and Hasselbalch (5) and others believed that there is a "work of development" (Entwicklungsarbeit), equivalent in birds and insects for the egg-incubation period, when including the maintenance cost, to about a third of the energy content of the unincubated egg. A similar belief was expressed by Terroine and Wurmser (6) for plant growth. Tyler (7) reported that the oxygen consumption (or energy liberation) by the differentiating sea urchin varies with the amount of differentiation.[1] Coleman and Du Bois (8) observed that the basal metabolic rate was 16 per cent above normal in adult typhoid patients when regaining weight during convalescence. It is, moreover, generally known that there is a pubertal metabolic acceleration coinciding with the growth acceleration (9). The pubertal metabolic acceleration may, however, be the result of increased endocrine activity rather than of rapid growth rate as such. This report on the energy increment of growth is confined to the suekling period, thereby avoiding, perhaps, metabolic changes due to endocrine activity.

Experimental. MacDowell and associates (1) working with mice reported maximum growth by redueing the number of young to four at birth, to two at 3 days, and to one at 5 days after birth. Working with Wistar-strain white rats, we observed best growth with reduction of the litter size to four at birth, to three at 2 days, and to two at 4 days. Reducing the litter to one rat sometimes resulted in failure to maintain the normal milk flow.

Oxygen consumption was measured on three average (7, 8, 9) litters, seven two-rat litters and one one-rat litter from birth until weaning at age 21 days.

The measurements were made in a Regnault-Reiset volumetric metabolism apparatus similar in type to the one deseribed by Winchester (10) for fowls. The animals were housed at 27°C. and measured at 30°C.

Results. Table 1 shows that the growth rates of the two groups are about the same until the fifth day when they begin to diverge, the weight eurve of the animals in the smaller litters surpassing that of the normal-size litters. The

[1] Collier reported an 85 per cent increase in oxygen consumption at the peak of regeneration of the fasting worm Tubifex tubifex (Jane Graybill Collier: The Relation between Metabolism and Morphogenesis During Regeneration. Doctoral Dissertation, University of Missouri, Columbia, 1942).

instantaneous percentage increases in body weight from the fifth day (when the average weights were the same for both groups) to the 21st day were[2] 158 per cent for the reduced litters and 108 per cent for the normals. The 50 per cent difference presumably reflects differences in milk supply to the two groups, the one whose growth rate was limited by the mother's lactational performance, and the other whose growth was limited by the inherent "growth-impulse" or food-intake capacity.

TABLE 1

Growth in weight and heat production of suckling rats, members of "normal" litters (7 to 9 per litter) and of reduced litters (1 to 2 per litter)

AGE, DAYS	AVERAGE WEIGHT, GRAMS		AVERAGE CAL. PER RAT PER DAY*		AVERAGE CAL. PER SQ. METER PER DAY**	
	Normal	Reduced	Normal	Reduced	Normal	Reduced
1	5.6	5.9				
2	6.2	6.1	2.12	2.15	611	625
3	7.1	6.6	2.31	2.36	611	654
4	8.3	7.8	1.99	3.43	552	855
5	9.7	9.7	2.70	3.13	587	680
6	10.6	11.5	3.11	4.54	639	887
7	11.6	13.2	3.48	5.20	680	930
8	12.8	15.2	2.98	7.43	544	1,216
9	14.2	17.4	2.55	6.85	436	1,035
10	15.4	18.8	3.22	7.39	520	1,059
11	16.3	21.8	2.97	8.16	466	1,064
12	17.3	24.1	3.41	8.69	394	1,064
13	18.8	24.5	4.05	7.00	580	848
14	19.8	28.2	4.05	8.49	561	941
15	20.8	31.2	4.23	9.34	569	960
16	21.7	33.8	4.74	9.84	620	973
17	22.9	36.8	4.52	10.01	571	939
18	23.5	39.4	4.73	11.31	588	1,016
19	24.4	42.5	4.83	11.34	587	972
20	25.3	45.4	5.29	12.22	628	1,004
21	28.8	47.3	6.60	12.78	722	1,023

* The Calories were computed from oxygen consumption on the assumption that 1 liter of oxygen is equivalent to 4.7 Calories.

** Surface area was computed from the equation surface in square meters = 0.0011 (weight in grams)$^{0.63}$ which is the average of the formulas by Carman and Mitchell (This Journal 76: 380, 1926), Lee (Idem. 89: 24, 1929), and Diack (J. Nutrition 3: 289, 1930).

Table 1 and figure 1 demonstrate a greater oxygen consumption by the more rapidly growing animals regardless of the reference base employed. Thus from figure 1, at body weight 20 grams, the metabolism of the more rapidly growing (smaller litter) animals is about twice as great as of the less rapidly growing (larger litter) animals. Some of this difference in heat production may be

[2] Computed by deducting the natural logarithms of the weights at age 5 days from that at age 21 days.

attributed to differences in specific dynamic action but not to the extent indicated. Some of this difference in heat production may be attributed to difference in "social-temperature regulation" (11) in small and large litters but not to the extent indicated. These data appear to favor the view that there is an organizational energy cost: the more rapid the growth rate, the greater the oxygen consumption or heat-production rate.

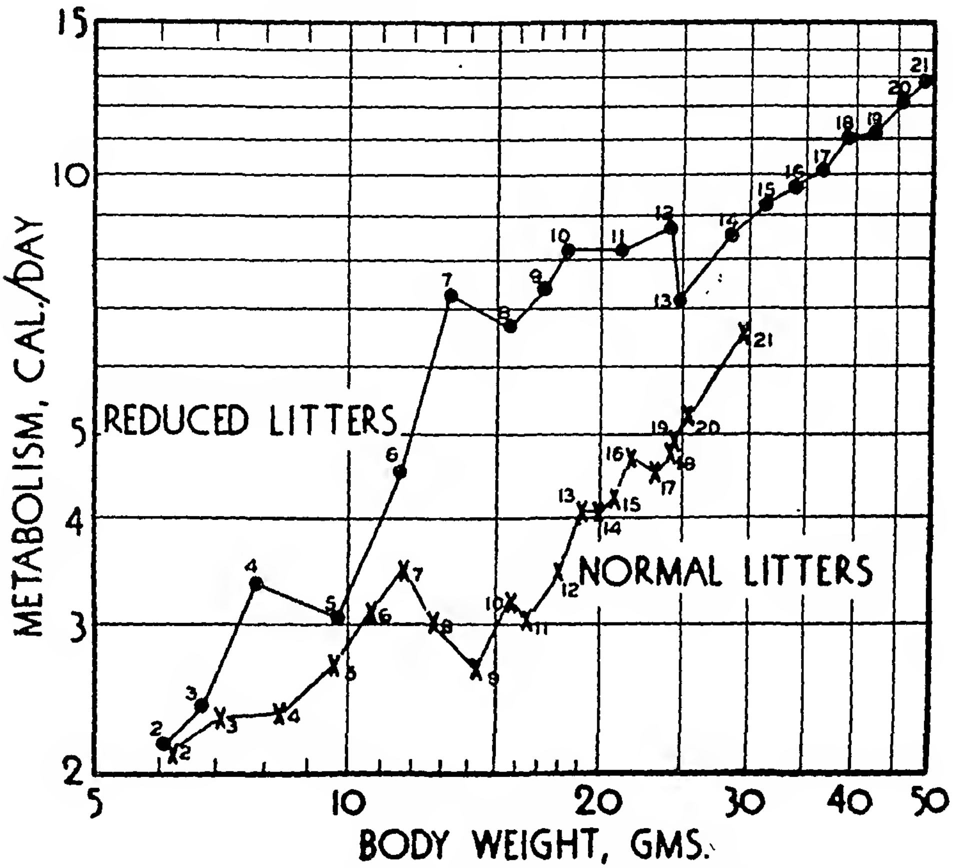

Fig. 1. Average metabolism in terms of Calories per rat per day as function of body weight of the rapidly and normally growing animals. The numerals on the curves represent ages in days counted from birth.

SUMMARY

This report presents daily averages on increase in weight and in oxygen consumption of suckling rats (up to 21 days), members of average-size litters (7 to 9 rats per litter), and of reduced litters (1 and 2 rats per litter). Beginning with the fifth day after birth, the average growth rate of the rats in the small litters was greater than that of the rats in the normal litters. The rapidly growing rats exhibited a higher heat production, regardless of the reference base employed, than did the normals. This is interpreted as evidence for the view that there is an organizational energy expense to growth and morphogenesis aside from the energy stored or other types of energy resident in the structure, as for example the energy of structural orientation postulated by Needham (12).

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THE EFFECT OF A PROTEIN-DEFICIENT DIET ON THE SERUM PHOSPHATASE AND HEPATIC DYE CLEARANCE OF DOGS

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Diets deficient in protein have been shown to produce changes in the function, structure, and possibly the permeability of the liver cell (1, 2, 3, 4, 5, 6). There is considerable evidence to indicate that an elevation of the serum phosphatase may be a sensitive indicator of certain alterations in liver function (7, 8, 9, 10, 11, 12).

The present study was undertaken to determine whether a protein deficiency would produce an elevation of the serum phosphatase of dogs. This question was answered in the affirmative. Next, a hepatic dye clearance test and the serum phosphatase were studied simultaneously in protein-deficient dogs to ascertain to what extent these two tests behaved similarly. It was found that the striking increase in the serum phosphatase produced by a protein deficiency paralleled closely the reduction in hepatic dye clearance produced by this deficiency. The changes observed in these tests are reversible and may be brought back to the normal range by adding one of the several so-called complete dietary proteins to the diet.

Methods. Adult mongrel dogs were used in all the experiments. The general procedure was to maintain the animals for about two weeks on an adequate diet during which time control values for serum inorganic phosphorus, serum phosphatase and hepatic dye clearance were obtained. Any animal showing abnormalities in appetite, activity or blood values was discarded. Some of the animals were then placed on the deficient diet, others on the control diet, and all were bled regularly at two week intervals for phosphorus and phosphatase determinations. In the third group of dogs hepatic dye clearance was also determined at two week intervals. When the animals became undernourished due to the gradual development of an anorexia they were sacrificed and immediately autopsied.

The protein-deficient diet which was fed at a level of forty calories per pound consisted of sucrose 55 per cent, lard 33 per cent, yeast (Anheuser-Busch Strain K) 5 per cent, Wesson's salt mixture 2 per cent, and ground cellophane 5 per cent. To each one hundred grams of diet were added 200 units of vitamin D and 1400 units of vitamin A in percomorph oil. The control diet was similar except that 25 per cent of the sucrose was replaced by protein in the form of either casein (both Labco vitamin-free and a commercial grade were used) or cooked egg white. The control dogs remained in a healthy state until the time they were sacrificed, which was between six and twelve months after the beginning of the experiment.

All blood determinations were made after at least twelve hours of fasting. Serum inorganic phophorus was determined by Bodansky's modification (13) of the Kutner-Lichtenstein method (14), using the Klett-Summerson photoelectric colorimeter. Serum phosphatase activity was determined by Bodansky's method (15). The method of Best, Channon and Ridout (1) was used to determine the total lipid content of the fresh liver. Hepatic dye clearance was measured by the Rose Bengal dye clearance test of Stowe, Delprat and Weeks (16). In using this test it was found convenient to use graded doses (0.14 cc./lb. of a 2 per cent solution in saline) and also to precipitate the proteins with eight parts of acetone to one part of serum. This gave filtrates which remained clear for long periods of time and could be conveniently read in the photolometer using a filter giving maximum transmission at 5400 Å. In using this test the time intervals were carefully controlled with a stop watch and it was found that the test could be repeated on the same animal with an accuracy greater than 90 per cent.

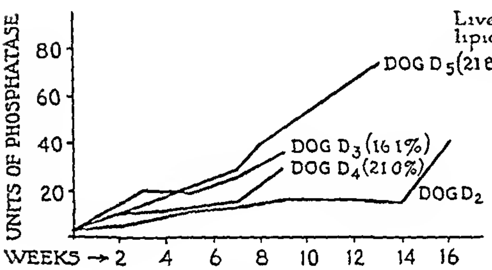

Fig. 1

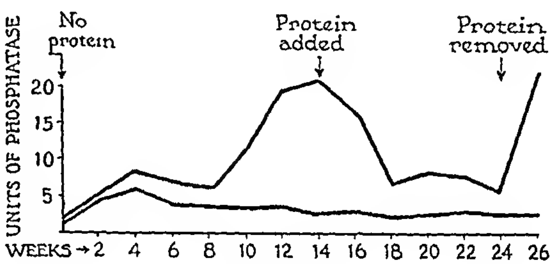

Fig. 2

Fig. 2. Upper line: average serum phosphatase of 5 dogs maintained on a protein-deficient diet with supplements as indicated.

Lower line: average serum phosphatase of 2 dogs maintained on protein-control diet. One per cent choline was included in the diet of this group from the 4th to the 6th weeks.

RESULTS. The gradual increase in the serum phosphatase which resulted from a protein-deficient diet is shown in figure 1. These four dogs are typical of the twenty-four protein-deficient animals which manifested this increase during 34 depletion periods. The control dogs receiving protein showed no significant increase in serum phosphatase. Normal adult dogs on an adequate diet usually have a serum phosphatase activity between one and four Bodansky units. The time required for an animal being depleted to show a rise above this range was found to vary from two weeks in some animals to eight weeks in others. The level to which the phosphatase activity rose also varied and seemed to depend to some extent upon the age of the animal, younger animals exhibiting the higher values. The increases observed in adult dogs have varied from ten to seventy-four Bodansky units per hundred cubic centimeters of serum. By the time the elevation of the serum phosphatase had reached what appeared to be a maximum the animals began to refuse a portion of their food, had lost 10 to 15 per cent of their body weight and in most cases had developed trophic ulcers on their extremities. Only two of the twenty-four treated dogs showed evidence of an

edema at the height of the first depletion. Some of the animals were sacrificed then and the fat content of the fresh liver was determined. The livers of D_3, D_4 and D_5 contained 16.1, 21.0 and 21.8 per cent fat respectively. A yellowish-brown pigmentation of the pancreas and small intestine was a conspicuous autopsy finding in many of the dogs. Chronic duodenal ulcers were found in four of the animals which had been depleted more than once. The liver cells were largely replaced by droplets of fat. The infiltration of fat was greatest about the periphery of the functional lobule of the liver. There was no significant increase in fibrous tissue in the livers of any of these animals.

In figure 2 is shown the result of returning protein to the diet of the depleted animal. Either casein or cooked egg white fed at a level of 25 per cent of the diet brought about a decrease in the serum phosphatase. The incomplete proteins, gelatin and zein were fed to a few animals, but because they would not

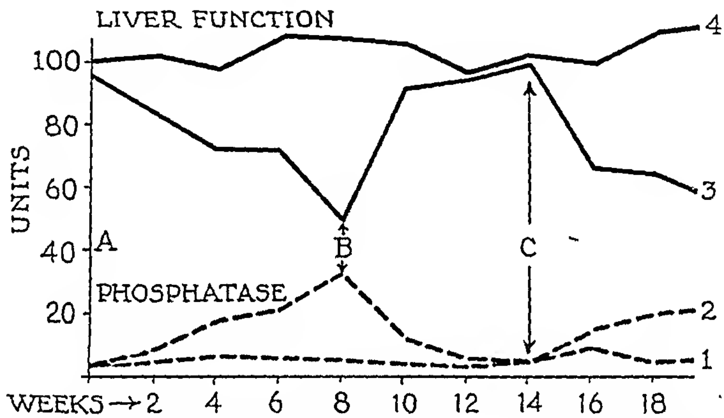

Fig. 3. *1*, serum phosphatase of 2 control dogs. *2*, average serum phosphatase of 3 treated dogs. *3*, average liver function of 3 treated dogs. *4*, liver function of 2 control dogs.

A, treated dogs started deficient diet. *B*, protein added to diet of treated dogs. *C*, protein removed from diet of treated dogs.

consume the diet in amounts adequate for maintenance the results are difficult to interpret and will not be considered here. When casein or egg white were added to the diet the phosphatase returned to essentially normal values in two to four weeks. However, changes in the general behavior of the animals were seen much sooner. A dog that had become emaciated and inactive during a protein-deficient regime and had reduced his caloric intake to as little as 50 to 100 calories per day, almost immediately began eating adequate amounts of food and became more animated. In two to three weeks the ten to fifteen per cent weight loss had been regained. After four weeks on the experiment this group received 1 per cent choline chloride in the diet for two weeks. This probably accounts for the longer time required to complete the depletion of the deficient animals as compared to those shown in figures 1 and 3.

In figure 3 the relation which exists between the increase in serum phosphatase and the decrease in hepatic dye clearance is shown. The upper solid line and

lower dotted line represent the per cent of normal dye clearance and serum phosphatase respectively of two control dogs. The middle two lines represent the hepatic dye clearance and phosphatase of three treated dogs. Eleven other dogs in fourteen experiments have given almost identical results. In practically every case where the phosphatase increased the hepatic dye clearance decreased and in most cases where the enzyme activity decreased, the dye clearance increased. However, the exceptions to this last generalization require an explanation.

Two of the animals which had been depleted to the point where their phosphatase had increased and their hepatic dye clearance had decreased were fed beef heart fractions as a supplement to the basal protein-deficient diet. The fractions used were kindly supplied by Dr. David Klein of the Wilson Laboratories and consisted of the residue from repeated hot water extractions of fresh beef hearts and the concentrated water extract. The residue or extractives derived from 2 grams of dry whole beef heart protein was fed for each pound of body weight. When fed the residue for six weeks a decrease from 9.1 units to 4.5 units of phosphatase and an increase in hepatic dye clearance from 72 per cent of normal to 93 per cent occurred in dog 1-E. Dog 5-E in six weeks exhibited a decrease in phosphatase from 47.2 units to 11.1 units and an increase in hepatic dye clearance from 35 per cent of normal to 63 per cent. Both these responses were positive ones, but the magnitude of the change was roughly only about two-thirds of the response a similar amount of casein or egg white would have brought about. When the concentrated water extract was fed in equivalent amounts there occurred in dog 1-E after two weeks a slight rise in phosphatase and a decrease in hepatic dye clearance from 80 per cent to 73 per cent. Dog 5-E showed a rise in phosphatase from 11.1 units to 21.3 units in 2 weeks and a decrease in hepatic dye clearance from 63 per cent of normal to 35 per cent. At this time the animal developed ascites and was estimated to have about two liters of fluid in his peritoneal cavity. One other animal was fed the extract but died suddenly before a second set of determinations could be made.

Discussion. With the exception of studies in which rachitogenic diets were fed, only a few experiments have been reported in which the serum phosphatase activity of dogs was shown to be influenced by the diet. Bodansky (17, 18) reported increases in the serum phosphatase of dogs after feeding fasted animals large amounts of carbohydrate. He also demonstrated that nursing puppies have a higher serum phosphatase activity than fasting puppies. These values were obtained on non-fasting blood and are believed by Bodansky to be due to the increased carbohydrate metabolism. Since the results being reported here were obtained on fasting serum they cannot be compared with the transitory rises observed by Bodansky. Freeman and Farmer (19) found that the fasting serum phosphatase values of dogs maintained on high protein diets (beef hearts) were consistently lower than values obtained on high carbohydrate (bread and meal) diets. The nature of the changes they observed are probably of a similar origin to those reported here.

Numerous studies (7, 8, 9, 10, 11, 12) have been made of the increase in serum phosphatase which occurs in certain types of liver damage other than the fatty infiltration due to a protein deficiency. None of these studies has, however, furnished indisputable evidence in regard to the origin of the serum phosphatase. The greater part of the evidence points to the liver as an important source, and seems to indicate that a liver slightly damaged or irritated by various factors produces increased amounts of the enzyme, but an extensively damaged liver cannot do this. Freeman, Chen and Ivy (12), in order to show whether or not the increase in serum phosphatase which results from a partial obstruction of the liver is due merely to the loss of excretory function, obstructed a lobe of the liver in several dogs and obtained a prolonged increase in the phosphatase activity. In a second group of dogs they removed an amount of liver tissue equal to that which had been obstructed in the first series and did not find a comparable increase in the enzyme in the serum. Schiffmann and Winkelman (20) have shown that the serum phosphatase elevation following obstruction of the common bile duct is greater in normal animals than in those previously poisoned with arsenic trioxide. Since this compound injures the liver, it may be inferred that the difference in serum phosphatase resulted from injury to the liver.

The experiments reported here provide no basis for determining the site of origin of the serum phosphatase. The changes occurring concomitantly in the liver, i.e., an increase in the lipid content, a decrease in the cell cytoplasm, and a decrease in the protein content (21) suggest that the increased phosphatase activity may be due to the loss of excretory function, alterations in cell permeability, or both. The decreased ability of the liver to remove intravenously injected Rose Bengal is a sensitive indicator of the excretory changes which are occurring. The possibility that the increased enzyme activity is due to a change in the concentration in the blood of an activator or inhibitor substance has not been ruled out.

The effect of the proteins, casein and egg white, in bringing about a decrease in the serum phosphatase and an increase in the hepatic dye clearance may be due to their lipotropic properties (1, 2, 3, 4, 5, 6). The lipotropic effect of proteins is now believed to be dependent upon the ratio of methionine to cystine in the dietary protein (22). Du Vigneaud et al. (23) have postulated that the lipotropic effect of methionine is due to the ability of this amino acid to furnish methyl groups for the synthesis of choline within the body, and that it is the choline which directly or indirectly brings about the reduction of liver fat.

It is well established that chemical, mechanical, and bacterial agents which injure the liver are capable of causing an elevation of the serum phosphatase. In the interpretation of abnormally high serum phosphatase values of unknown etiology one should also consider certain nutritional deficiencies which affect the liver, particularly those which may result in a protein deficiency.

SUMMARY

In 34 experiments on 24 dogs it has been demonstrated that the removal of protein from the diet causes an increase in the serum phosphatase and a decrease

in the hepatic clearance of Rose Bengal. These changes occur relatively early in the protein-deficient dog. The addition of protein in the form of either casein or cooked egg white will reverse these findings.

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THE RELATIVE RETENTION OF INFUSED CHLORIDE, UREA AND WATER

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The following study was undertaken to demonstrate experimentally that the kinetics of excretion of both threshold and no-threshold substances possess fundamental similarities. In the unanesthetized dog a constant infusion of known composition was administered (intravenously in these experiments) for several hours, and all urine was collected, generally at one hour intervals, and analysed. Computations were made merely from the complete input and output information, blood analyses being generally unnecessary.

Definitions. The hypothesis is made that rate of excretion of any substance is proportional to the load of the substance in the body; and the animal is regarded as being initially in a state of balance with no appreciable loads (with a qualification for urea noted under Procedures), that is

$$uU = \gamma L = \gamma(A - A_T)b \tag{1}$$

where u is rate of urine flow in cc./min.; U is concentration of a urine solute in mgm./min.; L is its load in mgm.; A is its plasma concentration in mgm./cc.; A_T is its renal threshold concentration in mgm./cc.; b is its volume of distribution in cc.; and γ is a "velocity constant" of excretion of the substance. This equation is based on the law of exponential decay, and is applied to all substances regardless of threshold. It is useful, however, to note the significance of the velocity constant for no-threshold substances. By definition,

$$C = \frac{uU}{A} \tag{2}$$

where C is clearance.

By definition
$$\gamma = \frac{uU}{L} = \frac{uU}{Ab} \tag{3}$$

Therefore,
$$\gamma = \frac{C}{b} \tag{4}$$

Thus the velocity constant simply represents the ratio of the clearance of a no-threshold substance to its volume of distribution. Since γ is the constant of proportionality of rate of excretion to load, it also is to be found in the integral form

$$A_t = A_i e^{-\gamma t} \tag{5}$$

where A_t is the plasma concentration at time t, and A_i is the initial plasma level, in the case of a decaying load. Then

$$\gamma = \ln\left(\frac{A_i}{A_t}\right) \Big/ t \tag{6}$$

and the velocity constant is seen as the slope of the line relating ln A to time. From equations (6) and (4) it is clear that Barnett's (1940) k value in connection with inulin clearances is the velocity constant of inulin excretion, representing the ratio of the inulin clearance to its volume of distribution.

Dominguez (1934) originally showed some of the significance of the velocity constant of excretion. However, the experiments to be considered here include the velocity constant not as a useful end in itself, but as a means to an end, viz., the study of relative retention and excretion. Emphasis is placed upon it as a variable parameter rather than as a constant.

From an infusion of known rate and from the known excretion up to time t, we can compute L (load) and the velocity constant. No plasma analysis is required.

The theory of the isorrheic state. In 1940 Eggleton, Pappenheimer and Winton employed the term "isorrheic" to describe constant urine flow in the isolated kidney. The meaning of this useful term is here extended by examining the properties of the isorrheic state and of isorrheic infusions, *in vivo*.

If the constituents of a solution infused at a constant rate could appear in urine with the same relative concentrations of solute and solvent that they have in the infused fluid, we might expect, in time, a load of infused fluid to build up to a point so that the isorrheic state (isorrhea) would be established, i.e.,

$$uU = iI = \text{a constant} \tag{7}$$

where i is the rate of infusion in cc./min. and I is concentration of infused fluid in mgm./cc. If the velocity constant remained constant

$$\frac{dL}{dt} = iI - \gamma L \tag{8}$$

and on integration

$$L_t = \frac{iI(1 - e^{-\gamma t})}{\gamma} \tag{9}$$

and from equation (3),

$$(uU)_t = iI(1 - e^{-\gamma t}) \tag{10}$$

and when t becomes large, uU approaches iI.

For many substances there is a value (an upper limit) of U above which a steady state cannot be maintained. That is, the kidney can elaborate urine of

increasing concentration of substance x from infusions of increasing concentration of substance x until a point is reached at which further increase of I_x results in no marked increase of U_x; but to satisfy the urgency of removing the load of x, increase of u occurs. The highest urine concentration which can be maintained without relative retention of x to water (y) may be called the *limiting isorrheic concentration* (L.I.C.) and denoted by $[U]$. With a constant infusion of a concentration set so that $I_x = [U]_x$, a load of x can be reached so that output will equal input, that is, isorrhea will be established and

$$u[U]_x = iI_x \tag{11}$$

but since

$$[U]_x = I_x \tag{12}$$

$$u = i \tag{13}$$

Therefore as a result of the infusion of x whose concentration equals the L.I.C. of x, both solute and solvent reach isorrhea simultaneously. It is implicit here that the excretion rates of solute and of solvent (x and y, respectively) reach the same fraction of their infusion rates at time t. Thus

$$\frac{uU_x}{iI_x} = (1 - e^{-\gamma_x t}) = \frac{uU_y}{iI_y} = (1 - e^{-\gamma_y t}) \qquad (14)^1$$

Therefore

$$\gamma_x = \gamma_y \tag{15}$$

Hence an important property of a L.I.C. infusion is that the velocity constants of solute and solvent are equal; or the entire infusate may be regarded as possessing a characteristic velocity constant. At concentrations of I not equal to U, it will be shown that the velocity constants of solute and solvent may be neither equal nor constant.

In the work presented here the usefulness of the velocity constant is quantitatively examined. The velocity constant of excretion is a constant only under special conditions; but so long as these are understood and controllable, the velocity constant is a useful tool in the study of relative retentions of different substances, concentration ceilings and related problems.

PROCEDURES. The bladder trigones of female dogs were exteriorized so that urine could be collected accurately and continuously while each dog stood in a stall and was infused with various solutions by ear vein at a constant rate for approximately 7 hours. The usual rate of infusion was 2.3 cc. per minute. Dogs suffered no appreciable discomfort during the procedure.

Blood from leg veins was drawn into tubes containing potassium oxalate at the beginning, middle and end of an experiment, and the plasma was analysed for chloride or chloride and urea.

The average rate of excretion during a time period was related to the loads at the end of that period, no attempt being made at interpolation since such correction was of little consequence to the final analysis of the data. Load was computed as the difference between the amount of a substance infused and

[1] It is assumed that $uU_y = u$ and $iI_y = i$.

the amount excreted by the end of a period. The average rate of excretion during this period divided by the load was called the velocity constant. The load of solute divided by the load of solvent (water) was called the *retention concentration* $\left(\frac{L_{\text{solute}}}{L_{\text{water}}}\right)$, and was a measure of the virtual concentration of the volume of infused fluid retained at the end of a time period, uncorrected for insensible loss of water. The ratio of the retention concentration to the infused concentration was called the *relative retention* $\left(\frac{L_{\text{solute}}}{L_{\text{water}} \cdot I}\right)$. So long as we neglect insensible water loss, if the relative retention were maintained at unity, the solute and solvent would be excreted at equal rates per unit load, i.e., $\gamma_{\text{solute}} = \gamma_{\text{water}}$. If the relative retention were maintained greater than unity, solute would accumulate relative to water ($\gamma_{\text{solute}} < \gamma_{\text{water}}$); and if the relative retention were maintained less than unity, water would accumulate relative to solute ($\gamma_{\text{solute}} > \gamma_{\text{water}}$).

Insensible water loss was estimated during the experiment from weighings of the dog and stall on a balance adapted to the purpose. The difference between fluid weight infused and sensible weight removed (urine and blood samples) differed from the balance-measured weight change in any time period by the insensible loss.

Animals were kept on no special diet. Daily variations in their salt and urea content apparently made little difference to the consequences of presenting them with a 7 hour load of infusate. Thus an animal was always regarded as having a zero salt and water load at the beginning of an experiment. In the case of urea, a no-threshold substance, the computation of the velocity constant was modified by adding to the infused load approximately 2000 mgm./17 kgm. body weight/20 mgm. per cent plasma level (pre-experimental) of urea. This inclusion of the normal body content of urea in the load of that substance is necessary if the velocity constant of urea is to have the same kinetic significance as the velocity constants of chloride and water.

Chloride concentrations are reported as milligrams of sodium chloride per cubic centimeter, although no implications concerning the behavior of sodium are intended. All the data presented below unless otherwise specified refer to the state of the animal at the end of a 7 hour infusion.

Results. Retention concentrations of chloride change with the duration of infusion (fig. 1). The most significant fact, perhaps, is the tendency of all curves below 17 mgm./cc. at zero time to approach the 6 mgm./cc. retention concentration asymptotically. And all curves above 17 mgm./cc. proceed to higher values without limit. That 6 mgm./cc. is an asymptote will be shown presently. Commonly the initial trend of the retention concentration (when below 17 mgm./cc.) is the opposite to that displayed later, but this offers little hindrance to attaining the 6 mgm./cc. value because relatively small volumes of urine are excreted initially (compared to later ones) and the infused loads in early periods are likewise relatively small.

Table 1 is a protocol indicating in detail how excretion rates, velocity constants, retention concentrations, etc., vary with the duration of infusion.

Figures 2 and 4 relate the velocity constants of water and chloride, and water and urea, respectively, to infusion concentrations (urea infusions contained NaCl in concentration of either 6 or 17 mgm./cc., approximately). Despite the gross variability of the data plotted, the velocity constant of water tends to go through a minimum (fig. 2) and the velocity constant of chloride rises linearly. The higher value of I (17 mgm./cc.) at which the velocity constants of water and of chloride are equal is the *limiting isorrheic concentration* (L.I.C.). The lower value of I (6 mgm./cc.) at which these velocity constants are equal may be called the *non-limiting isorrheic concentration* (NL.I.C.).

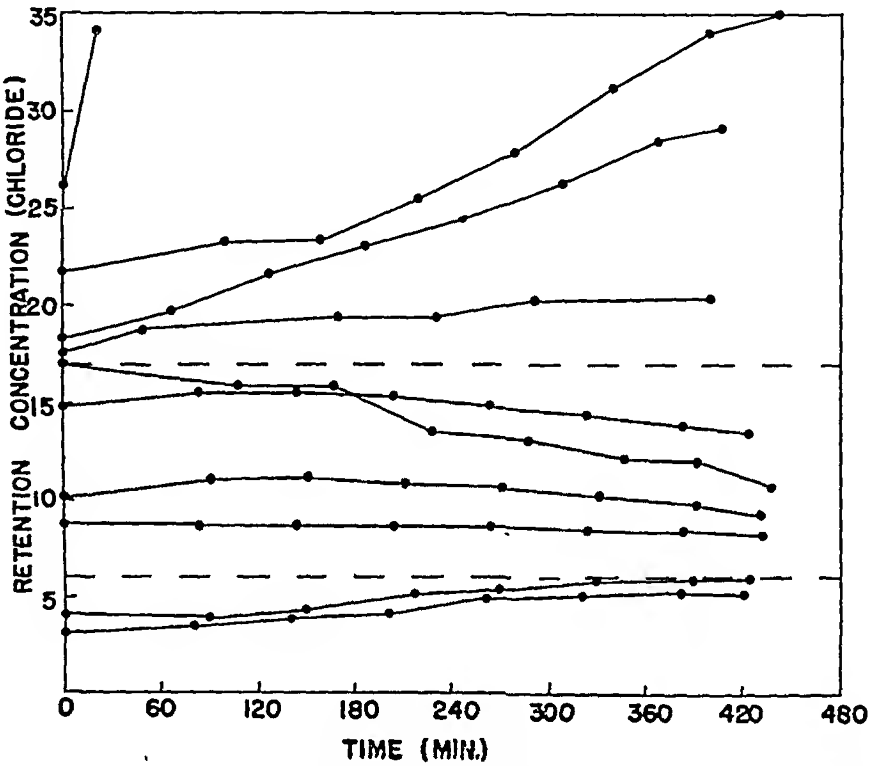
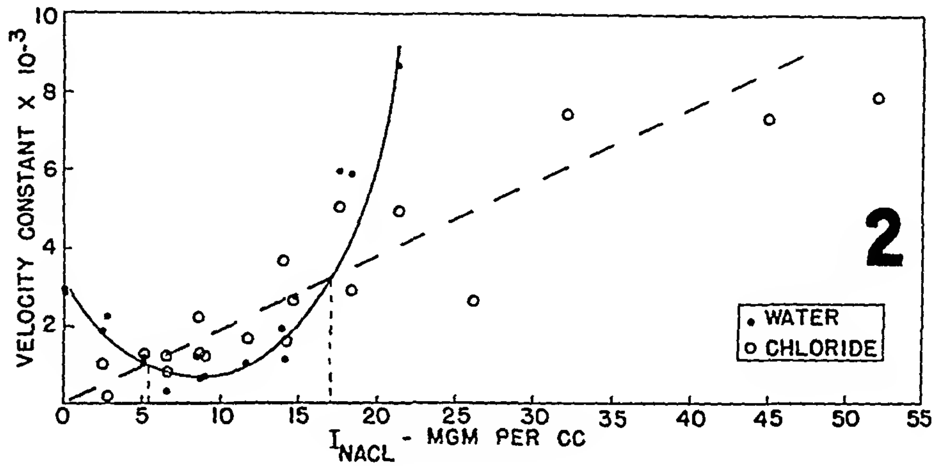


Fig. 1. Relative retention in relation to time of infusion. The experiments illustrate the tendency of an infusion of chloride of concentration less than 17 mgm./cc. (upper horizontal broken line) to be retained in time as 6 mgm./cc. (lower horizontal broken line). Infusion concentrations greater than 17 mgm./cc. are not tolerated indefinitely.

The velocity constant of urea (fig. 4) is rather constant after an initial "augmentation" concomitant with the increased rate of urine flow at 7 hours at high I_{urea} values as compared with low I_{urea} values. A L.I.C. of urea is evident at about $I = 35$ mgm./cc. There is no NL.I.C.

The urine concentrations also change with I (figs. 3 and 5) to bring about these relations. When I_{Cl} is less than 6 mgm./cc., U_{Cl} is always less than I_{Cl}, and so the velocity constant of chloride is less than that of water. When I_{Cl} is greater than 6 and less than 17 mgm./cc. (neglecting insensible loss for the present), U_{Cl} is greater than I_{Cl}. Thus the velocity constant of chloride is greater than that of water. When I_{Cl} is greater than 17 mgm./cc. the urinary

chloride concentration does not increase appreciably under the conditions of these experiments and the velocity constant of chloride is less than that of water, so that the relative retention of chloride increases with increasing duration of infusion. U_{urea} is always greater than I_{urea} below the L.I.C. so that the



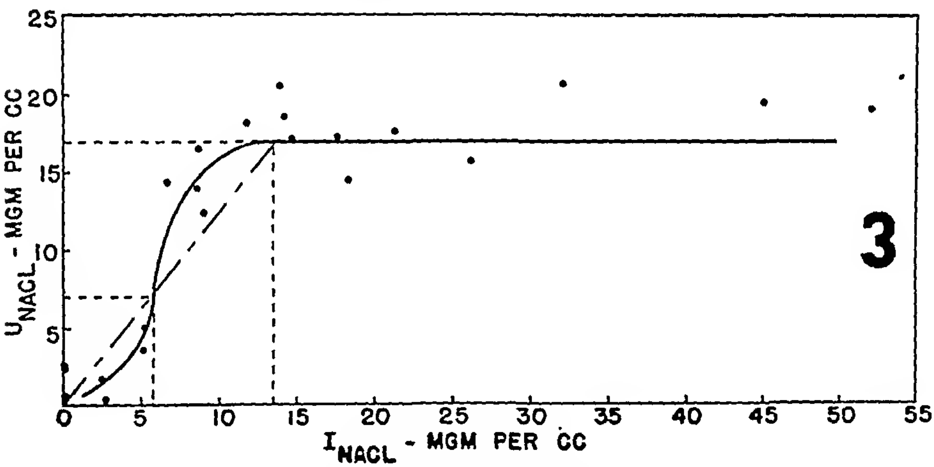


Fig. 2. Values found for velocity constants in relation to infusion concentration after 7 hours of infusion at 2.3 cc./min. The points of intersection of the curves represent the non-limiting isorrheic concentration and the limiting isorrheic concentration. Dogs 5 and 6. Wts. 18 and 10 kgm., respectively.

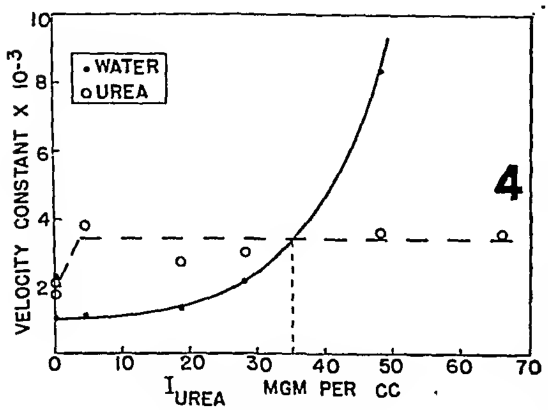
Fig. 3. Urinary concentration of chloride in relation to infused concentration of chloride. The broken line rising from the origin has a slope of 1.2 which corrects for insensible water loss. Dogs 5 and 6.

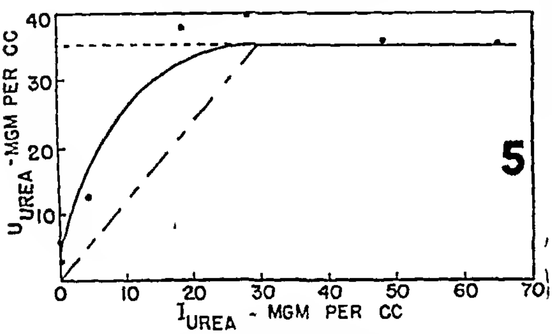
velocity constant of urea is greater than that of water below this value of 35 mgm./cc.

The relative retention of chloride as a function of I_{Cl} at the end of 7 hours is presented in figure 6. Curve $AA'A''$ based on the experiments of figure 2 indicates that at infusion concentrations of less than 6 mgm./cc., chloride is retained relative to water. This is true also above 17 mgm./cc. Between these critical points (NL.I.C. and L.I.C.) water is retained relative to chloride.

From figures 2 and 6 it follows that if the relative retention were maintained at unity, the velocity constants of water and chloride would be equal.

A similar relation holds for urea (fig. 8) although having only one critical point (a L.I.C.) the relative retention of urea is unity at one point only. This graph indicates that the L.I.C. is about 27 mgm./cc. The plot of retention





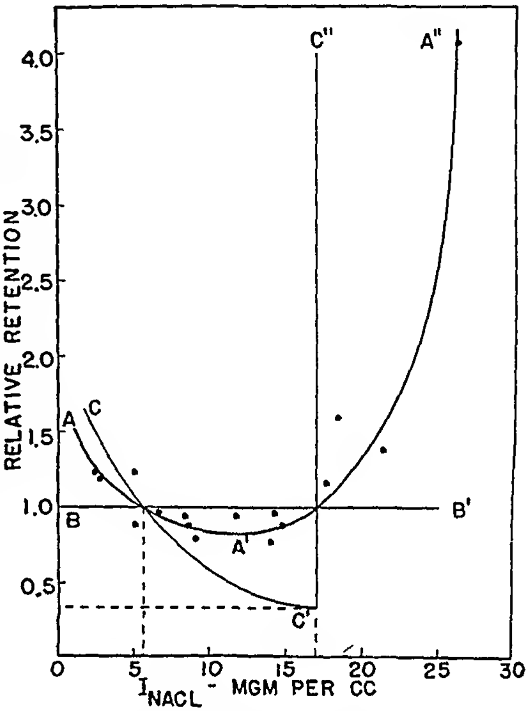


Fig. 4. Values found for velocity constants in relation to infusion concentration after 7 hours of infusion at 2.3 cc./min. Infusion contained 6 mgm. of chloride per cubic centimeter. There is only one point of intersection, i.e., only a limiting isorrheic concentration. Dog 5.

Fig. 5. Urinary concentration of urea in relation to infused concentration of urea. The broken line rising from the origin has a slope of 1.2 which corrects for insensible water loss. Dog. 5.

Fig. 6. Relative retention of chloride as a function of I_{Cl}. Curve $AA'A''$ is for the 7th hour determinations. Curve BB' is for zero time. Curve $CC'C''$ is for infinite time. All intersect mutually at critical points, i.e., at the non-limiting isorrheic concentration and limiting isorrheic concentrations of chloride. Dogs 5 and 6.

concentration against I_{urea}, also in figure 8, indicates 30 mgm./cc. as the L.I.C. (where infusion and retention concentrations are equal). The value of 35 mgm./cc. for the L.I.C. of urea is probably a better one as is suggested by figures 4 and 5.

Figure 7 illustrates a useful relation from which the L.I.C. and NL.I.C. may be drawn. By combining pairs of velocity constants of water and chloride as a ratio in a single experiment, critical values of 6 and 17 mgm./cc. may be estab-

lished for those retention concentrations at which the ratio of the velocity constants is unity.

All the seventh hour findings on chloride and water not represented in the various graphs are collected in table 2. Corresponding figures on urea and water (and chloride) are presented in table 3. These tables extend the observations to other dogs, and support the inferences drawn from the graphs.

It was noted after several hours of infusion of chloride of about 80 mgm./cc. concentration, that signs of salt intoxication (Guttman, 1865) were evident in tremor of the legs and head, increased restlessness and a thirst, presumably exquisite, judging from the fact that the act of turning on the water faucet within

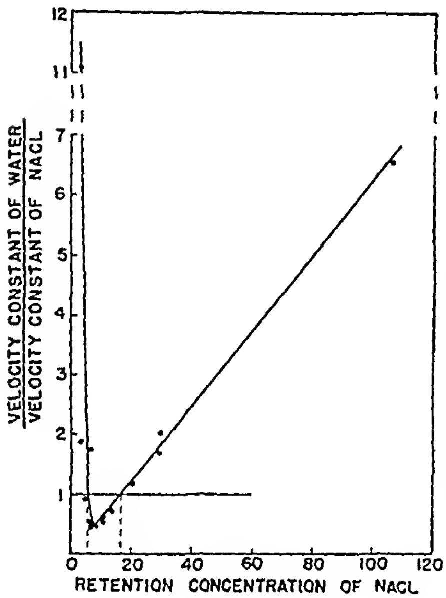


Fig. 7

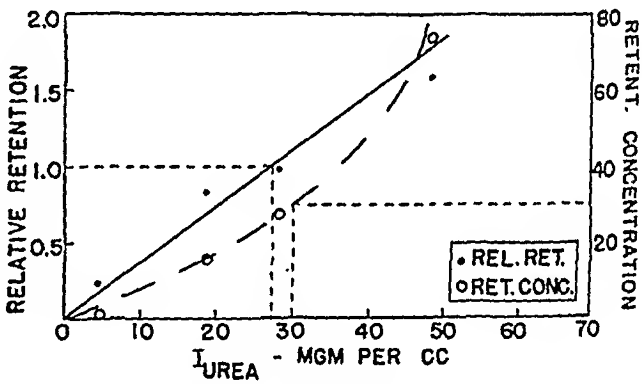


Fig. 8

Fig. 7. Non-limiting and limiting isorrheic concentrations are those retention concentrations of Cl at which the velocity constants of chloride and water are equal (i.e., when their ratio is unity). Dogs 5 and 6.

Fig. 8. Retention concentration and relative retention of urea as a function of infusion concentration of urea. Dog 5.

sight of the dog was sufficient to excite it violently. Such infusions were stopped before 7 hours for fear of killing the dogs. The velocity constants of chloride and water began to fall rapidly in the later, violent stages, perhaps indicating the onset of serious injury. Dogs having salt intoxication will, if they retain sufficient motor control, drink water (or their urine) greedily until they are forced to vomit. Then they drink further. The solution to their problem seems to be to excrete the excess salt in due time rather than to dilute it all at once, the latter process probably calling for many liters of water. The dogs recovered from this treatment with no evidence of permanent injury.

The results indicate that the dog seeks to retain infused chloride at a virtual concentration of 6 mgm./cc., excreting relatively more chloride than water from more concentrated infusates, and excreting more water than chloride from less

concentrated infusates. The dog cannot successfully continue to eliminate chloride from infusions more concentrated than 17 mgm./cc. of chloride, or to eliminate urea from infusions more concentrated than 35 mgm./cc. of urea. Dehydration (negative water loads) and relative retention of solute to water set in, increasing with the duration of such concentrated infusions.

Urea excretion obeys the law of exponential decay rather exactly over wide ranges of its concentration in infusates. Chloride and water excretion do not simply follow this law of decay except in cases of infusion with I_{Cl} set at 6 or 17 mgm./cc.; but they vary with the infusion concentration of chloride as well as with their respective loads.

Insensible water loss. In these experiments where the rate of infusion was generally about 2.3 cc./min., the factor of insensible loss was found to be appreciable. For dog 5, this averaged 0.37 cc./min. and for dog 10, about 0.43 cc./min., or approximately 16 per cent and 19 per cent of the infusion rate, respectively.

TABLE 1

A protocol of experiment 64 (table 2)

I_{Cl} = 3.1 mgm./cc.

TIME	INFUSED CHLORIDE	EXCRETED CHLORIDE	LOAD OF CHLORIDE	EXCRETION RATE OF CHLORIDE	VELOCITY CONSTANT OF CHLORIDE	L_{Cl}/L_{H_2O}	RELATIVE RETENT. OF CHLORIDE	PLASMA CONC.	INFUSED WATER	EXCRETED WATER	LOAD OF WATER	EXCRETION RATE OF WATER	VELOCITY CONSTANT OF WATER
min.	*mgm.*	*mgm.*	*mgm.*	*mgm./min.*	*min.*$^{-1}$	*mgm./cc.*		*mgm./cc.*	*cc.*	*cc.*	*cc.*	*cc./min.*	*min.*$^{-1}$
88	055	142	513	1.61	0.00314	2.88	0.93		211	33	178	0.376	0.00211
148	1100	296	804	2.56	0.00319	2.98	0.96	7.0	356	86	270	0.884	0.00327
208	1550	460	1090	2.78	0.00256	3.09	1.00		500	148	352	1.03	0.00294
268	1990	610	1380	2.42	0.00175	3.08	0.99		644	194	450	0.767	0.00170
328	2440	730	1710	2.09	0.00122	3.24	1.04		787	260	527	1.10	0.00209
388	2890	920	1970	3.16	0.00160	3.43	1.10		931	355	576	1.58	0.00274
428	3180	1070	2110	3.94	0.00187	3.57	1.15	6.4	1030	440	590	2.07	0.00350

Thus the L.I.C. of urine may not be so low as the L.I.C. of infusion fluid and U and I may never be maintained equal to one another. The broken line rising from the origin in figures 3 and 5 has a slope of $i/(i - w)$ where w was taken as about 20 per cent of i. Above and below this line U is to be found at the end of an infusion of finite duration. Theoretically as the relative retention approaches unity with increased time (when I is less than 17 mgm./cc.), all points will lie on such a line.

Discussion. *The velocity constant as a function of infusion concentration.* The relatively constant urea velocity constant which is found above an apparent "augmentation" limit (fig. 4) regardless of I_{urea} or I_{Cl}, is a measure of the fact that the law of decay accurately describes the excretion rate of this substance. However, the linear rise of the chloride velocity constant with I_{Cl} (fig. 2) gives no such description. Since

$$\gamma_{Cl} = KI_{Cl} \tag{16}$$

where K is a constant, then from (3)

$$uU_{Cl} = KL_{Cl}I_{Cl} \tag{17}$$

Equation (17) states that rate of chloride excretion is proportional not only to load but also to infusion concentration. It might be held that the law of

TABLE 2

Seventh hour data from infusions of chloride without urea

Dog weights: 10, 15 kgm.; 11, 16 kgm. Data not appearing in the graphs are to be found in this table.

DOG NO.	TIME	SUBSTANCES	I	TOTAL INPUT	LOAD	EXCRETION RATE	VELOCITY CONSTANT	L_{Cl}/L_{H_2O}	RELATIVE RETENTION	H_2O/Cl VELOCITY CONSTANT RATIO	PLASMA CONC.
	min.		*mgm./cc.*	*mgm. or cc.*	*mgm. or cc.*	*mgm. or cc./min.*	*min.*$^{-1}$	*mgm./cc.*			*mgm./cc.*
10	425	NaCl	0.0	0.0	0.0	0.76					5.8
		H_2O		994	530	2.55	0.00481				
10	428	NaCl	3.1	3180	2110	3.94	0.00187	3.57	1.15	1.87	6.4
		H_2O		1030	591	2.07	0.00350				
11	416	NaCl	3.1	2980	2700	1.53	0.00057	3.51	1.13	3.62	6.9
		H_2O		963	767	1.53	0.00200				
10	424	NaCl	3.5	3480	1470	4.94	0.00336	3.28	0.94	1.21	6.9
		H_2O		992	449	1.83	0.00408				
10	425	NaCl	4.0	3800	3440	1.25	0.00036	5.93	1.48	5.40	6.5
		H_2O		983	582	1.14	0.00196				
10	428	NaCl	7.0	7070	4910	12.0	0.00244	7.30	1.04	1.26	6.4
		H_2O		1010	673	2.07	0.00308				
10	432	NaCl	10.5	10600	6840	19.4	0.00284	9.35	0.89	0.57	6.9
		H_2O		1010	731	1.18	0.00162				
10	424	NaCl	14.8	14500	7100	39.3	0.00554	13.5	0.92	0.79	7.3
		H_2O		980	525	2.30	0.00437				
10	438	NaCl	17.1	17400	3820	39.4	0.0103	11.7	0.68	0.62	8.4
		H_2O		1020	326	2.06	0.00638				
11	440	NaCl	21.7	22600	8260	41.2	0.00499	35.1	1.62	1.94	7.3
		H_2O		1040	235	2.27	0.00965				
10	420	NaCl	24.8	24800	10400	60.0	0.00580	46.9	1.89	2.43	7.8
		H_2O		1000	221	3.11	0.0141				
10	429	NaCl	36.6	36200	7190	96.0	0.0134	−15.4	−0.42	−0.77	8.4
		H_2O		991	−467	4.80	−0.0103				
11	408	NaCl	49.5	46900	10500	110.0	0.0104	−12.0	−0.24	−1.79	9.0
		H_2O		946	−877	5.14	−0.00585				

decay actually applies here as with urea, but that another factor, I, modifies the expression of the law. What would be the influence of I_{Cl} on the chloride velocity constant? Why should the rate of excretion of chloride at $I = 17$ mgm./cc. be three times greater than when $I = 6$ mgm./cc., for a given load of chloride?

TABLE 3

Seventh hour data from infusions of urea (and chloride)

DOG NO.	TIME	SUBSTANCES	I	INPUT	LOAD*	EXCRETION RATE	VELOCITY CONSTANT	L_{solute}/L_{H_2O}	RELATIVE RETENTION	H_2O/Cl VELOCITY CONSTANT RATIO	H_2O/UREA VELOCITY CONSTANT RATIO	PLASMA CONC.	INCREMENT OF PLASMA CONC.
	min.		*mgm./cc.*	*mgm. or cc.*	*mgm. or cc.*	*mgm. or cc./min.*	*min.*$^{-1}$	*mgm./cc.*				*mgm./cc.*	*mgm./cc.*
5	422	NaCl	2.8	3080	2320	0.47	0.00020	3.32	1.19			7.8	
		H_2O		1060	699	1.55	0.00222			11.1	10.5		
		Urea	0.0	0	2000	4.21	0.00211	0				0.204	−0.016
5	415	NaCl	5.1	5050	3450	4.16	0.00121	4.50	0.88			6.3	
		H_2O		989	768	0.84	0.00109			0.902	0.613		
		Urea	0.0	0	2800	4.98	0.00178	0				0.208	−0.120
5	423	NaCl	5.9	5640	3880	8.15	0.00211	5.16	0.88			5.9	
		H_2O		953	750	0.87	0.00115			0.545	0.300		
		Urea	4.7	4400	2840	10.9	0.00384	1.11	0.24			0.36	0.14
10	409	NaCl	6.0	5190	1610	8.55	0.00533	2.36	0.394			7.9	
		H_2O		868	680	0.60	0.00089			0.166	0.100		
		Urea	9.06	7850	2740	24.1	0.00882	−0.39	−0.043			0.54	0.12
5	410	NaCl	5.8	5370	3090	8.15	0.00264	4.43	0.76			6.1	
		H_2O		925	697	0.96	0.00137			0.520	0.492		
		Urea	18.8	17300	13000	36.2	0.00279	15.8	0.837			0.84	0.61
5	425	NaCl	6.0	5750	2590	9.27	0.00357	4.43	0.74			7.0	
		H_2O		956	586	1.27	0.00217			0.607	0.710		
		Urea	28.1	26900	16600	50.8	0.00306	27.8	0.990			1.18	0.93
10	428	NaCl	6.4	5750	1610	14.5	0.00900	4.43	0.691			6.2	
		H_2O		900	365	2.45	0.00672			0.747	0.990		
		Urea	33.8	30400	11300	76.5	0.00680	17.1	0.507			1.30	0.79
5	407	NaCl	4.6	4120	1030	11.8	0.0115	3.44	0.748			7.1	
		H_2O		917	299	2.50	0.00837			0.729	2.32		
		Urea	48.1	44000	24700	89.4	0.00361	75.7	1.57			1.84	1.53
5	402	NaCl	7.0	6250	1680	16.2	0.00963	−120	−17.2			8.8	
		H_2O		854	−14	3.23	−0.231			−24.0	−64.3		
		Urea	65.9	56100	31700	114	0.00360	−2120	−32.2			2.64	2.45
10	417	NaCl	6.4	6550	−1010	25.3	−0.0252	3.69	0.58			7.6	
		H_2O		1021	−273	4.44	−0.0162			0.643	−0.284		
		Urea	75.5	77300	30700	175	0.00570	−112	−1.49			3.45	3.12
10	422	NaCl	17.0	17500	5700	30.8	0.00541	11.5	0.675			8.1	
		H_2O		1030	496	1.45	0.00292			0.540	0.707		
		Urea	9.18	9460	4530	18.7	0.00413	2.07	0.226			0.470	0.06
10	414	NaCl	17.6	17000	5450	38.6	0.00708	13.0	0.740			8.5	
		H_2O		994	419	1.97	0.00471			0.666	1.13		
		Urea	19.1	19000	9790	40.8	0.00417	17.4	0.910			0.833	0.530
10	429	NaCl	16.7	17200	3250	42.5	0.0131	−37.4	−2.24			8.0	
		H_2O		1030	−87	3.31	−0.0381			−2.91	5.57		
		Urea	51.6	53100	18200	124	0.00684	−179	−34.6			2.13	1.81

* In case of urea, the value given for "load" actually represents the load plus the normal body content.

Distortion. It seems feasible, for descriptive purposes, to interpret the increase of the chloride velocity constant with increasing I_{Cl}, when no other solute is in the infusion fluid, independently of direct consideration of glomerular filtration rate.

Consider the volume of distribution of water in the body divided by a semipermeable membrane into a chloride and a non-chloride space, b_{Cl} and b_d, respectively. Assuming the system a perfect osmometer, regard the concentration of chloride in b_{Cl} as A_{Cl}. At equilibrium, the effective concentration in b_d may also be called A_{Cl}. It can be shown from such considerations that upon the addition of a load of chloride solution to the chloride space

$$\frac{I_{Cl}}{A_{Cl}} = f\left[\frac{\left(\frac{b'_{Cl}\, b_d}{b'_d}\right) - b_{Cl}}{L_{water}}\right] \tag{18}$$

where b'_{Cl} is the new chloride space, b'_d is the new non-chloride space, and f represents "a function of."

This expression indicates that the ratio I/A is a measure of the departure from normal of the relative sizes of chloride and non-chloride space per unit volume of infusate. Whether the animal acts as a perfect osmometer is not an important question here. Within limits it seems like such a system (Lands, Cutting and Larson, 1940). This ratio, for some purposes may be called the *distortion* of the chloride space, and as a number, indicates by its magnitude the degree of imbalance of the normal relations of volume of the chloride to the non-chloride space. The product of the distortion and L_{water} represents some function of the *distorted volume.* The discrepancy between such a theoretical distorted volume and the real erratic volume is unimportant so long as some type of volume shift is induced by chloride infusion. An infusate containing 6 mgm. chloride per cubic centimeter induces a distortion of nearly 1. A pure water infusion would induce no distortion of chloride space.

A tenable hypothesis seems to be that although the excretion rate of a substance is ordinarily proportional to load, the kidney might be obligated not only to discharge loads the more rapidly as they are the greater, but also to maintain the normal ratio of the solute and non-solute volumes of distribution. This concept has been considered by Peters (1942). In the case of chloride excretion, the distortion factor may be regarded as at least equal in influence to concentration or load factors. Urea should show no distorting influence since its volume of distribution is common with that of water. Without distortion, the law of exponential decay is revealed for urea in a relatively uncomplicated fashion. The unknown way in which a distortion factor operates, if it has significance at all, may make suspect its case for recognition as an entity. How a distorted volume could influence the nephrons of the kidney is unknown unless it be through changes in the kidney's own intraspatial relations.

It is believed that the excretion rate of chloride, when it is infused with no other solute, may be related to two factors: 1, load, and 2, distortion. The latter is an expression of the departure from normal of the relative volumes of chloride and non-chloride spaces and is a function of the ratio I/A. An increase

of distortion (increase of I/A) is concomitant with an increase in the excretion rate of chloride per unit load of chloride. If excretion rate were simply proportional to super-threshold concentration, then infusions of $I = A_T$ might never be excreted since the super-threshold concentration $(A - A_T) = O$. This supports the contention that a load can be excreted at a rate related to the distorted volume which it creates. In contrast, the excretion rate of urea seems to depend only on load (above an augmentation limit). Since urea and water occupy approximately the same volume of distribution, the urea space is not distorted appreciably. This lack of a distortion factor for urea is in harmony with the nicety with which urea excretion apparently obeys the law of exponential decay.

The velocity constant as a function of infusion duration. The kidney apparently operates to convert the volume of infused fluid to a retained concentration of 6 mgm./cc. Thus we may view the NL.I.C. of 6 mgm./cc. as a sort of "physiological saline" elected by the animal and guarded by the kidney.

One of the most important factors in determining the shape of the curve $AA'A''$ (fig. 6) is the duration of the infusion. Thus at zero time after infusion, the retention concentration must be equal to the infusion concentration since no time has elasped for the transformation of the infusate by excretion. And the curve BB' is the theoretical curve obtained at zero time; the relative retention is unity independently of I_{Cl}. If an infusion proceeded for infinite time, an infusion of concentration just short of 17 mgm./cc. would have been converted to a retention concentration of 6 mgm./cc. and the relative retention would be 6/17 or 0.35. This would be the lowest possible relative retention under these conditions. If the infusion concentration exceeds 17 mgm./cc. then at infinite time, the retention concentration would become infinite and the relative retention would be infinite. Any I value less than 17 mgm./cc. should in infinite time give rise to a relative retention of $6/I$ and thus the curve $CC'C''$ is derived. It may be observed how the 7 hour curve $AA'A''$ fits between the zero time and infinite time curves.

Thresholds. Attention is called to the concept of thresholds by the finding of the tendency to retain a "physiological saline." Rehberg (1926) suggested that thresholds ought to be defined only for actively resorbed substances. Using a reference substance (creatinine) at that time presumed to be neither secreted nor resorbed by tubules, he found that when chloride fell below about 6.2 mgm./cc. in the plasma, active resorption occurred (since the concentration of chloride in the resorbed fluid was higher than the plasma concentration), and above 6.2 mgm./cc. no active resorption was apparent and chloride acted like a no-threshold substance. This is sensibly the same conclusion arrived at in the present work, although only chloride analyses of urine and infusion fluid are required by the technique. The present work supports the rationale of the Rehberg threshold, further defining it as a NL.I.C. of chloride, or a plasma concentration above which chloride is excreted relative to water and below which chloride is retained relative to water.

The difference between the maximum and limiting isorrheic concentrations of urine. That U can ever exceed its L.I.C. brings up the question as to why the L.I.C.

should be different from the maximum urine concentration. A partial answer to this may be ventured, although it is probable that a great deal of evidence remains to be gathered on this matter. This answer concerns the notion that if the infusion of urea which would ordinarily bring about a given U_{urea} value, had contained sufficient chloride to induce a greater urine flow at the end of some period, the U_{urea} would be expected to have a smaller value so that the rate of excretion of urea for the same load of urea would be a constant. The rise of U on a falling urine flow is also consonant with maintaining a constant urea velocity constant although the reasons for the rapid decline of the velocity constant of water under these conditions is not fully clear. Urine containing as much as 25 mgm./cc. chloride has been found, a deviation from the L.I.C. of 47 per cent.

SUMMARY

1. A technique for studying the relative retentions of constantly infused solutes and water is described, and theoretical aspects of the isorrheic state (constant urine flow or solute excretion with constant infusion rate) are developed.

2. The velocity constants of excretion (rate of excretion per unit load) of chloride, water, and urea have been ascertained in the dog. They may be used to characterize those critical concentrations of infusion fluid and urine at which neither solute nor water are retained from an infusion, relative to one another. For chloride, expressed as sodium chloride, these concentrations are approximately 6 and 17 mgm./cc. For urea the only such concentration is approximately 35 mgm./cc. (when the simultaneously infused chloride is less than 17 mgm./cc.) under the conditions of these experiments.

3. The law of exponential decay is regarded as the underlying pattern followed by the excretion of threshold as well as no-threshold substances, and factors which relate to the modification of this pattern are examined. Excretion rate of chloride can be expressed not only as a function of load but also as a function of the distortion, or departure from normal, of the ratio of chloride to non-chloride space, induced by this load. The excretion of urea is uncomplicated by a distortion factor.

4. Rehberg's concept of threshold concentration for ions like chloride is extended and a simple technique for determining and expressing such threshold concentrations is suggested.

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THE BEHAVIOR OF THE SPLEEN IN HEMORRHAGIC HYPOTENSION AND SHOCK[1]

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In cases of death from shock, the spleen at autopsy has been reported as small, firm, dry, contracted or anemic, as well as large, soft and congested. In experimental shock following intestinal exposure, Y. Henderson (1) noted distention of the spleen. Erlanger and Gasser (2) describe the spleen as "often enlarged and containing hemorrhagic areas" after caval occlusion, aortic clamping and intestinal exposure. Whipple, Stone and Bernheim (3) mention splenic congestion in some of their dogs that died from intestinal obstruction. Moon (4), on the contrary, insists that the organ contains less blood than normally and in the dog is dry, firm and contracted. Lindgren (5) who used the Sjödstrand method for evaluating blood volume of organs by staining red corpuscles only, reported reduction in blood content of the spleen in guinea pigs that died from histamine or operative shock. However, they used spleens of decapitated animals (hemorrhage!) as a criterion of the normal. Zwemer and Scudder (6) described a red and leathery spleen in dogs that died from traumatic shock. Moore (7) who determined the spleen/body weight ratios of cats, shocked in various ways, found that the ratios agreed with the smallest values previously reported by Barcroft for normal cats. In reviewing the autopsy protocols of 48 experiments reported upon by Werle, Cosby and Wiggers (8), we find that the spleen was described as small, hard or maximally contracted in thirty-nine, normal or slightly contracted in six, and large or engorged in three experiments. However, the difficulty of deciding how large a spleen ought to have been if the animal had died a natural death often made decisions difficult in the instances in which it was classed as "normal."

Since the spleen shrinks at death, regardless of the manner in which this occurs (Barcroft, 9), observations of spleen size made post mortem cannot be expected to yield much information with regard to its size during life. For these reasons, we studied the changes in the size of exteriorized spleens during the course of hemorrhagic hypotension and hemorrhagic shock by the method of Barcroft and Stephens (10).

Methods. Dogs were anesthetized with morphine and sodium barbital as in previous studies (8). Subclavian pressures were recorded optically by calibrated optical manometers of the Gregg type and by turning a 3-way cock mean pressure could also be read on the calibrating manometer. A femoral artery was cannulated for bleeding and a femoral vein for reinjecting the blood.

The spleen was exteriorized through a small midline incision and laid on a flat surface, taking care not to exert tension or torsion on its pedicle. It was loosely

[1] This investigation was supported by a grant from the Commonwealth Fund.

covered with cellophane to prevent drying. Its contour was traced with a wax pencil on a glass plate held in juxtaposition to the spleen and the outline so obtained was transferred to paper. The area was measured with a planimeter. Frequent duplicate outlines and measurements were made as checks. Since, for our purpose, nothing was gained by translating surface areas into probable volumes as described by Barcroft and Stephens (10), our results are reported in terms of area and the changes as percentage variations from the controls before bleeding. These were obtained after completion of operative procedures and allowance of a 20 to 30 minute interval for attainment of circulatory equilibrium.

When control sizes remained uniform, the dogs were bled at a moderate rate (ca. 100 cc./6min.) until mean arterial pressure was reduced approximately to 50 mm. Hg. The animal was allowed to recover for $\frac{1}{2}$ to 1 hour and a second slower hemorrhage continued until the pressures were reduced to 40 mm. or below and held there for 45 minutes to 2 hours, the exact time depending on the animal's resistance and the object of the experiment. When necessary, small quantities of blood were reinfused to maintain viability of the respiratory center. After the period of hypotension, the total volume of withdrawn blood—prevented from clotting by addition of liquaemin[2] (Roche) and properly strained—was reinjected, as in experiments previously reported (8). The subsequent course of events depended on the intensity and duration of the hemorrhagic hypotension.

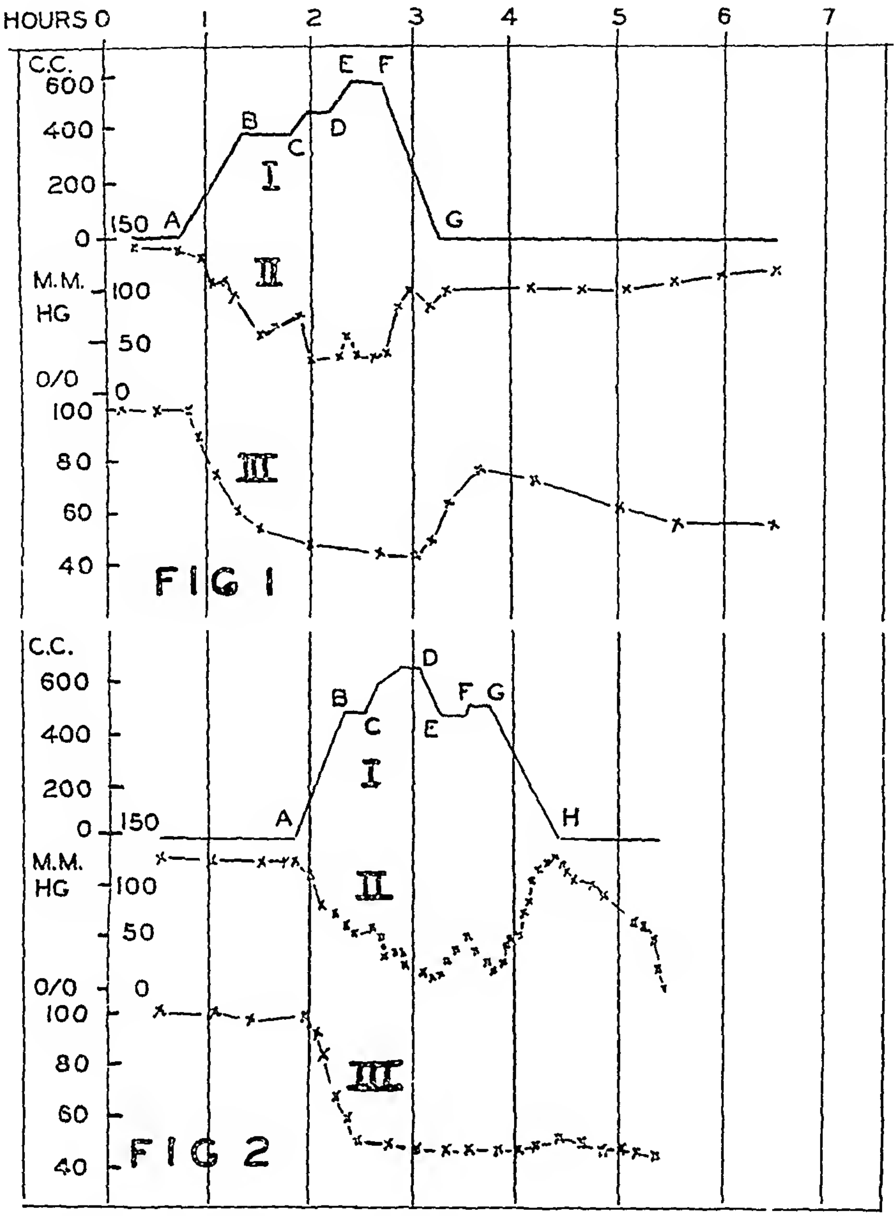
Results. It should be stated that, in a control experiment on a dog under similar morphine-sodium barbital anesthesia, the size of the exteriorized spleen remained practically constant for a period of six hours, and during the ensuing $2\frac{1}{2}$ hours gradually decreased to 90 per cent of these values.

In order to study the effects of a brief period of extreme hypotension, two experiments were performed in which this lasted 45 minutes only. Then all the blood was reinjected. Results from one of these are shown in figure 1. After withdrawal of 366 cc. at *A-B*, mean arterial pressure was reduced to 54 mm. and the optical pulses showed all the characteristics of a severe hemorrhage, previously described (8). The spleen size decreased to 57 per cent of the control and continued to decrease to 49 per cent during the compensatory rise of pressure to *C*. At this time, the animal was bled an additional 90 cc. and later, *D*, an additional 100 cc. This maintained arterial pressures approximately at 30 mm. for 45 minutes. The spleen contracted further to 43 per cent of its control size. It should be noted that the greatest decrease occurred during the initial hemorrhage which confirms results of Barcroft et al. (10) and Grindlay et al. (16).

With reinfusion of all the withdrawn blood *F-G*, mean arterial pressures recovered to 100 mm. Hg. For $3\frac{1}{2}$ hours thereafter arterial pressure pulses of good form were maintained and the mean pressure gradually rose. The spleen increased to 78 per cent of its normal size during reinfusion, but thereafter, and without significant changes in arterial mean pressures or pressure pulses, it gradually decreased again to 55 per cent of the control. The animal was sacri-

[2] We are indebted to Roche-Organon, Inc., Nutley, New Jersey, for their generous supply of the liquaemin solution used in these experiments.

ficed at the end of $6\frac{1}{2}$ hours and at autopsy revealed no gross pathological changes, including the duodenal mucosa.



Figs. 1–2. Curve *I*, volume of blood withdrawn upward, reinfusion downward. Curve *II*, changes in mean arterial pressure. Curve *III*, percentage changes in area of exteriorized spleen.

Figure 2 shows results on an 18 kgm. dog. The mean blood pressure at the beginning of the experiment was 125 mm. Hg. After a control period of 90 minutes *A*, blood was withdrawn from the animal at a fairly uniform rate of 100 cc. per six minutes until the blood pressure had dropped to 55 mm. Hg, *B*.

The total amount of exsanguinated blood at this time was 500 cc. The animal was allowed to equilibrate at this pressure for a period of fifteen minutes. The respirations during this period were increased in both rate and depth. At *C-D* an additional 150 cc. of blood was removed and the mean blood pressure fell to 20 mm. Hg. The spleen area decreased progressively during the initial hemorrhage, but not much more during the second bleeding. When the mean blood pressure reached 20 mm. Hg the respirations were very shallow, heart action was feeble and it was thought advisable to reinject 200 cc. of blood in order to save the animal, *D-E*. As a result of this injection, the mean blood pressure gradually rose to 50 mm. Hg, but the spleen area did not show a corresponding increase. In order to maintain a pressure level less than 50 mm. Hg, the dog was bled another 25 cc. at *F*.

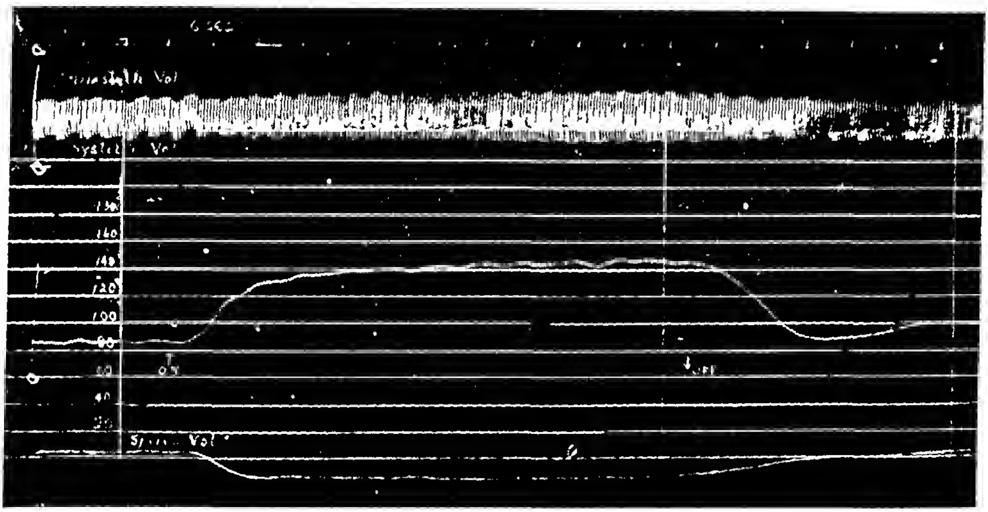


Fig. 3. Tracings showing effect of stimulating a central vagus nerve on spleen volume (lower), mean arterial pressure (middle), and stroke volume of the ventricles recorded by an oncometer and segment capsule (upper). Note changes in diastolic size (upper part of cardiometer record).

After an approximate $1\frac{1}{2}$ hour period of severe hypotension the remaining blood was reinjected at *G* and the mean blood pressure rose to 135 mm. Hg. The pressure pulses were large and reasonably normal as regards contour. However, the spleen showed only a slight tendency to increase in size and never approached its control value. This mode of behavior differs materially from that observed during the reinfusion period in figure 1. Shortly after the reinjection the mean blood pressure started to fall, the pressure pulses became smaller and began to deteriorate. One hour later, when mean pressure had been reduced to 50 mm. the animal expired in respiratory failure. It is of interest to note that the spleen area slowly decreased somewhat more during this final period of progressively developing hypotension, but never significantly below the minimum value reached during the initial hemorrhage. This can be interpreted to mean that during hemorrhage the spleen contracted to its maximum ability and could react to no further stimulus of hypotension.

At autopsy the duodenal, jejunal and ileal mucosa was swollen, edematous, purplish and intensely congested. Hemorrhagic areas were present in the mucosa and the lumen contained extravasated blood. The characteristic sharp demarcation at the duodenal bulb described previously by others (2, 3) and by us (8) was present. The spleen was dry, pale and rough, with a few small irregular hemorrhagic areas beneath its capsule. No blood drained from the cut surface and little could be squeezed out by pressure.

The results of six other experiments were similar to those detailed above. In all cases the spleen area decreased rapidly and extensively during a hemorrhage sufficient to induce serious hypotension and contracted slightly more during the period of low tension. This indicates that, in the dog, the splenic contraction not only acts as a compensatory mechanism to place more blood into circulation during loss of blood volume but remains contracted during the period of post-hemorrhagic hypotension as well. When blood was reinjected the spleen enlarged somewhat, but never to control size, if the period of hypotension had not been too prolonged. Thereafter it slowly decreased again (cf. fig. 1) and apparently aided in sustaining the venous return and arterial pressure in animals that did not develop circulatory failure within three or four hours after reinfusion. In animals that developed hemorrhagic shock according to dynamic and pathological criteria, the spleen remained contracted during reinfusion (fig. 2) or increased very slightly for a short time. A dilated spleen, or one that approximated the control size, was never observed in these experiments.

Finally, we performed two experiments to determine whether greater concentration of blood might modify the results. To this end, the blood withdrawn was centrifuged and the corpuscles reinjected with a small amount of Locke's solution. A typical shrinking of the spleen occurred with each hemorrhage and a slight recovery (ca. 8–10 per cent) followed the injection of red cells. The injection of heparinized plasma after a period of hypotension sufficient to induce circulatory failure did not affect the spleen size; indeed, in one experiment it decreased still more toward the end, i.e., to 54 per cent of the control size.

Discussion. The dynamic significance of splenic contraction and its purpose in hypotensive states merit brief discussion. Changes in the size of the spleen have been frequently recorded in physiological and pharmacological investigations and several points of view have apparently developed as to the inferences which may be drawn from such changes in size.

One viewpoint appears to be that changes in spleen size permit conclusions regarding changes in peripheral resistance and particularly those in the splanchnic area. Our own experiences indicate that such deductions are not allowable. From a hydraulic viewpoint, the spleen circuit is only a minor shunt in the many parallel circuits between the aorta and venae cavae (cf. fig. 3, ref. 11) and, *a priori,* changes in its resistance could have no great effect on total peripheral resistance calculated as $1/R = 1/r_1 + 1/r_2 + 1/r_3 + 1/r_4 \cdots 1/r_n$, in which the spleen circuit could be any one of the fractions. The truth of this deduction can be demonstrated easily in a dog under barbital anesthesia, i.e., under conditions in which the spleen is large and congested and its vascular resistance would

presumably be low. Clamping the whole pedicle of such an exteriorized spleen, i.e., making the splenic resistance infinite—is without noticeable effect on mean blood pressure or on optically recorded pressure pulses. In a few unreported experiments by Dingle, Kent et al. (12) in which T.P.R. was calculated on the basis of cardiac output and mean arterial pressure, no change was found on clamping the splenic pedicle. The inference would appear to be that changes in resistance in the splenic shunt, like those in the limbs (13), are essentially without effect on total peripheral resistance in the whole animal.

The assumption made by many, but specifically in relation to shock by Moore (7), that significant decrease in size of the spleen is an index of intense vasoconstriction throughout the splanchnic area, seems rather hazardous on *a priori* grounds. In experimental animals, such as the cat and dog, changes in spleen size and vascular resistance to flow through the organ are chiefly, if not wholly, determined by action of extravascular muscle fibers in trabeculae, not to muscular elements in arterioles, as in the rest of the splanchnic circuit. To conclude, without evidence, that activity of such extravascular musculature is necessarily co-ordinated with vascular muscle tissue elsewhere is indeed an assumption. That it is not permissible seems clear from other observations from this laboratory (14, 15) that calculated T.P.R. may be reduced in certain forms of shock, although the spleen is always greatly contracted, postmortem.

As emphasized by Barcroft (9, 10), the chief function served by contraction of the spleen in the dog and cat is to demobilize blood, i.e., to cause an autotransfusion and so increase venous return. That this is sufficient to improve venous return, increase the diastolic size of the heart and augment systolic discharge is shown by experiments, one of which is reproduced as figure 3. In this instance, splenic contraction and elevation of arterial pressures were produced by stimulation of pressor fibers of the central vagus. Similar and even more marked effects follow injection of epinephrine, etc. In all such reactions, contraction of the spleen contributes to the elevation of arterial pressure not by the increase in resistance, but by augmenting venous return and increasing cardiac output.

Consequently, the development of splenic contraction during posthemorrhagic hypotension and its maintenance despite restoration of blood volumes by blood infusion indicates that in dogs splenic contraction is one of the important compensatory mechanisms invoked to increase the diminished venous return.

The fact that the spleen remains contracted when an irreversible circulatory state develops, and indeed remains so until death, exhonorates the spleen as a factor which precipitates the irreversible state in shock.

SUMMARY AND CONCLUSIONS

In nine experiments on dogs, the changes in areas of exteriorized spleens were studied by the method of Barcroft and Stephens during hemorrhage, posthemorrhagic hypotension and hemorrhagic shock following reinjection of the blood which had been withdrawn.

In confirmation of previous reports, it was found that the spleen contracts

rapidly and extremely during hemorrhage, the area being reduced by as much as 50 per cent or more. In extension, it was found that during a period of prolonged posthemorrhagic hypotension the dog's spleen undergoes a further slow contraction, does not increase on reinfusion of the withdrawn blood, but remains contracted whenever the duration and intensity of the hypotension is sufficient to create dynamic and pathological signs of shock. Similar changes occurred in plasmapheresis experiments.

In the dog, splenic contraction does not contribute to elevation or maintenance of arterial pressure by virtue of the increased resistance induced in the splenic shunt, but by augmenting venous return and cardiac output. The spleen is not an organ which withdraws blood from active circulation in hemorrhagic shock. When the spleen is found large and congested at autopsy other factors must have operated.

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A STUDY OF SPONTANEOUS FULMINANT SHOCK IN A HEART-LUNG-DOG PREPARATION[1]

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Previously, Wiggers and Werle (1) had attempted to assess the relative importance of changes in venous pressure, myocardial response and total peripheral resistance in hemorrhagic shock[2] by registering changes in arterial and venous pressures and in ventricular volume optically and by subsequently calculating total peripheral resistances (TPR). Results strongly suggested that, in different animals, depression of the myocardium and failure of peripheral resistance contributed in different degrees to the production of an irreversible state. However, changes in cardiac output primary to myocardial depression and secondary to altering venous pressures could only occasionally be dissociated during the course of such experiments. It seemed possible that such evaluation might be accomplished continuously by producing shock in a preparation in which venous pressure and cardiac output were under control of the experimenter. In addition, changes in total peripheral resistance (TPR) could be studied in a preparation in which cardiac ouptut per minute was measured by a different method. For these purposes, we devised a heart-lung-dog preparation.

The heart-lung-dog preparation. This preparation resembled Starling's heart-lung preparation as regards artificial control of venous pressures and cardiac output, but retained the natural circulation of the animal and kept operation of its natural cardiac and vasomotor reflexes intact. The principle (fig. 1) consisted *1*, in draining blood returned both by the superior and inferior venae (*SVC*; *IVC*) into a low-level reservoir of about 500 cc. capacity; *2*, in pumping this blood by a rotary pump, *P*, to a Mariotte bottle, *M*, the overflow of which returned to the low-level reservoir, and *3*, in feeding the right heart exclusively by blood at constant temperature from the Mariotte bottle. Venous inflow was measured periodically by a stromuhr of the Ludwig type, *S.U.*, and this flow was considered equivalent to left ventricular output. The stromuhr was actuated by large electromagnetic clamps in circuit with a chronoscope, thus eliminating possible variations in reaction times inherent in any manual method of timing flows.[3] The principles of the perfusion scheme and the registration of aortic pressure, *OM*, and venous pressure, *VP*, should be obvious from the sim-

[1] This research was supported by a grant from the Commonwealth Fund.

[2] Hemorrhagic shock was differentiated from hemorrhagic hypotension by the production of intensive congestion, edema or even hemorrhage in the mucosa of the upper intestine and failure of the circulation to respond more than temporarily to reinfusion of the blood withdrawn.

[3] We are indebted to Dr. Harold Green for aid in designing the electromagnetic stromuhr.

plified diagram of figure 1. However, a few technical details must be mentioned:

Experience soon showed that 2500 to 3000 cc. of undiluted, noncoagulable and compatible dog's blood were required for such shock experiments. Of this, 1000 cc. were needed to load the perfusion system. Hence, three large dogs were bled using 0.3 cc. liquaemin per 100 cc. as an anticoagulant for drawn blood. The experimental animal was given an initial injection of 0.3 cc./kilo liquaemin and, after perfusion had started, 0.1 cc./kilo was added to the reservoir every 30 minutes.[4] In some experiments, Calcomine fast pink[4], 80 to 150 mgm. per kilo of the total weights of all animals was added, in addition.

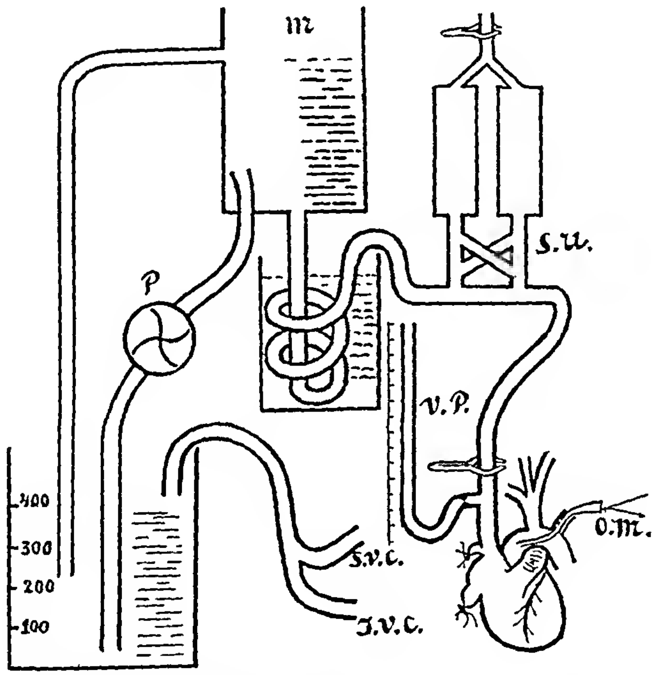

Fig. 1. Schematic diagram of heart-lung-dog preparation. Lettering explained in text. Not drawn to scale.

These exorbitant doses of liquaemin and dye were found necessary in order to preclude coagulation absolutely; smaller quantities invariably resulted in deposits of a fibrin on filters or caused infarct areas in the lungs, endocardium, mesentery or other organs.

The question at once arose as to possible deleterious effects of such enormous doses of anitcoagulants, particularly upon endothelial cells. Control injections of identical doses of liquaemin generally caused no hemodynamic changes; and only occasionally a temporary reduction of arterial pressures with a prompt recovery to normal. Similarly, the dye occasionally invoked temporary Traube-

[4] We are indebted to Roche-Organon Co., Nutley, N. J., for their generous contribution of the considerable quantities of Liquaemin solution required for these experiments and to Calco Chemical Co., Bound Brook, N. J., for a supply of Calcomine fast pink.

Hering fluctuations. After four or six hours, however, no signs of circulatory failure or pathological changes in organs could be discovered. The congestive or hemorrhagic changes in the intestinal mucosa characteristic of shock were never seen. Of course, when dye was used, the red color was generally distributed through tissues, serous fluids, bile, urine, etc. We must realize, nevertheless, that use of such enormous doses of anticoagulants may summate with other insults in making the capillaries more vulnerable to any tested shock-producing agent. They have, however, an advantage over defibrinated blood in that the blood used undergoes much less hemolysis and never evokes pulmonary edema.

The sequence of operative procedures in routine experiments was as follows:

Stage I. Cannulation of a femoral artery and vein—institution of a degree of artificial respiration which still allowed spontaneous contractions of respiratory muscles—opening of the chest without hemorrhage, meticulous attention being given to ligation of all vessels—exposure of the heart which was left within the pericardium—recording of mean femoral pressure—injection of liquaemin or liquaemin plus dye.

Stage II. Placement of a single ligature around the azygos vein, double ligatures around the inferior and superior cavae, and the left subclavian artery (for insertion of aortic manometer cannula).

Stage III. While femoral mean pressure was continuously recorded, the following procedures followed as quickly as possible: *a*, ligation of the inferior vena cava near the right auricle and insertion of a cannula into its peripheral end, allowing blood to drain into the low-level reservoir;[5] *b*, ligation of the superior vena cava peripherally and insertion of cannula into the vein near the right auricle—commencement of artificial perfusion of heart—insertion of a cannula into the peripheral end of the superior vena cava and drainage of its blood into the low-level reservoir; *c*, ligation of the azygos vein.

With ligation of the inferior vena cava mean arterial pressure fell approximately to 40 mm. Hg and on subsequent ligation of the superior cava to 15 or 20 mm. Hg. The heart continued to beat regularly but feebly on the small supply from the azygos vein until the perfusion was started. Artificial venous inflow and pressure were slowly increased until arterial mean pressure oscillated around 120 mm. Hg. The total duration of significant hypotension (50 mm. Hg or less) required for transfer to an artificial circuit varied from five to nine minutes; in most experiments it was 5 to 7 minutes.

Optical manometers were inserted after the perfusion system was operating properly. The temperature of the blood was kept constant at 36° to 38° in different experiments by a thermostatic control of the water bath surrounding the warming coil. Changes in temperature due to variation in flow were quickly rectified or anticipated by addition of cold or warm water to the bath and resetting of its thermoregulator. Tests on a number of preparations showed that vasomotor and cardiac reflexes were active during the start and course of the experiment (e.g., central vagus stimulation and carotid compression).

[5] As a rule, the animal yielded about 250 cc., ca 25 per cent, of its calculated blood volume by the time artificial perfusion was started.

Expectations and realizations. It was our expectation that, after the technique had been mastered, such a preparation would maintain an unaltered status for a number of hours and that the rôle of peripheral factors, venous pressure and cardiac impairment could be assessed when shock was willfully produced in different ways. We discovered, however, that even under the most propitious circumstances, our animals quickly showed clear evidences of a rapidly developing shock, such as deterioration in the form of arterial pressure pulses, consumption and storage of large quantities of the blood perfusate and exhibition of marked congestive and hemorrhagic changes in the upper intestines. In short, it was only necessary to follow the changes which occurred spontaneously. The process progressed so rapidly as to constitute a *fulminant type of shock.*

Such experiments stress the fact that shock is not necessarily a slow progressive chain of events, but under propitious circumstances can develop with extreme celerity and intensity. Perhaps technical improvements, not obvious to us, could still be made which would prevent development of such a rapid peripheral circulatory failure; but since each experiment involved the use of four dogs and large quantities of heparin, we chose to discontinue these studies with the completion of twenty-four such experiments.

Results. Before discussing illustrative experiments, the initial circulatory state of our animals should be assessed. Our experimental procedures previous to perfusion had been so far refined that our animals, with open chests and under artificial respiration often had mean pressures of 150–170 mm. Hg and pressure pulses suggestive of a mild hypertension. After the brief period of hypotension incident to a change from the natural to the artificial blood supply, venous pressures and cardiac output were so adjusted that mean arterial pressures of 100 to 140 mm. Hg were re-established. The venous pressures and cardiac output required to accomplish this varied considerably in different animals of approximately the same weight. This is shown in table 1.

Such outputs are definitely below values usually accredited to dogs of these sizes. The amplitude and form of aortic pressure pulses before and after artificial perfusion also indicated that the systolic discharge was less than previous to perfusion. Since the normal dog's heart has a larger cardiac output at effective venous pressures of 50 to 60 mm. H_2O than the best in our series and since some of our hearts delivered still smaller volumes per minute at even higher venous pressures (cf. expts. 4, 8, 13, 14), it would be a fair inference that such artificially perfused hearts are below par at the start. This of course applies to any heart-lung preparation. On the other hand, in all experiments, vigorous regular beats were resumed at once and the right ventricle appeared full but not dilated; in short, the hearts seemed in good condition by every observable criterion and, during initial periods of observation, responded with constant increments and decrements of output to definite elevations and reductions of venous pressure. In short, the myocardial depression which appeared with artificial perfusion was stabilized, or, in some instances, tended to decrease during the control period.

Since mean pressure was sustained at 120 mm. Hg, or above, on such small

cardiac outputs, and an inordinate hypertension developed when cardiac output was significantly increased, it is a fair inference that these animals were in a state of superconstriction at the start. This is also supported numerically by

TABLE 1

EXPT.	WT.	V.P.	C.O/MIN	T.P.R.	B.P.	EXP.	WT.	V.P.	C.O/MIN	T.P.R.	B.P.
	kgm.	*mm.* H_2O	*cc.*	*A.U.* $\times 10^3$	*mm. Hg*		*kgm.*	*mm.* H_2O	*cc.*	*A.U.* $\times 10^3$	*mm. Hg*
1	12.5	105	1167	9.6	140	10	12	60	882	10.15	122–114
										11.05	
2	13.0	83	1220	9.5	130–145						
				8.55		11	14.5	85	892	8.8	100–112
										10.0	
		52	848	12.66	125–135						
				11.75		12	12.5	92	994	10.46	130
						13	10.5	80	616	14.25	110
3	14	52–56	1017	10.54	120–135						
				9.43				120	855	11.02	122
4	14	60–65	781	13.2	115–130	14	10	108	906	7.67	87
				11.6							
						15	13	70	1055	9.1	120
5	14	32	608	18.29							
				13.13	100–140			50	658	12.0	100–112
										13.5	
6	12.5	43	527	18.0							
				15.17	100–120	16	16	67	1120	8.55	120
7	14	55	1139	8.22	102–118	17	12.5	60	650	13.75	112–122
				7.1							
8	13.5	66	743	11.09	92–105						
				9.7							
9	12	65	991	9.23	107–115						
				8.6							

the rather high values obtained when total peripheral resistance (TPR) was calculated in absolute units (A.U.) according to the formula:

$$\text{TPR} = \frac{\text{mean pressure} \times 1332}{\text{cardiac output/sec.}} = \frac{\text{dynes}\cdot\text{sec.}}{\text{cm.}^5} \text{ (A.U.)}.$$

While it is hazardous, at the current state of our knowledge, to draw too many conclusions from comparisons of calculated total peripheral resistance (TPR) made in different groups of experimental dogs, table 2 may have some interest.

This compilation indicates that the ranges of TPR calculated in this series are the highest of any reported series, and as shown in table 1, most of these

are near the upper ranges. The magnitude of the differences may be more intelligible when it is recalled that Dingle, Kent et al. (3) reported an experiment in which an increase in TPR from approximately 6000 to 15,000 A.U. represented the degree to which TPR augmented after a dose of 2 cc./1:50,000 adrenalin in a vagotomized dog. On the other hand, it should also be mentioned that, in repeated tests, stimulation of the central vagus or injection of neosynephrine, with constant cardiac output, were capable of increasing TPR enormously, showing that regardless of the high values they were certainly not maximal (see fig. 4).

While the significance and interpretation of changes in TPR, calculated in this customary fashion, deserve further study we are, for the time, accepting the interpretation that our preparations probably started with an intensive vasoconstriction and high TPR.

Shock without reduction in venous pressure. It is commonly postulated that all forms of shock have one feature in common, viz., initiation by a primary reduction in venous return. While there is substantial proof for the correctness of this doctrine in most forms of shock, some evidence exists that reduced arterial resistance may be the primary factor in certain types (for review and discussion, see 4). We shall first discuss representative experiments

TABLE 2

Böger (2)	4020–4470 A.U.*	probably small dogs
Dingle, Kent et al. (3)	3750–11,300 A.U.	(8–12 kilos)
Wiggers and Werle (1)	3931–11,714 A.U.	(10–15 kilos)
This series (see table 1)	7100–18,290 A.U.	(11–13 kilos)

* A.U. = absolute units in dynes. sec./cm^5.

which indicate that such a sequence occurs in the type of experiment which we designed.

Figure 2 graphically summarizes observations on a 14 kilo heart-lung-dog preparation in which right auricular pressure was kept constant at 55 mm. H_2O and the temperature of the blood remained at 37.5° throughout the experiment. The vagus nerves and the carotid arteries were intact. The period of hypotension caused by transfer of the heart from the natural to the artificial blood supply lasted six minutes. Ten minutes after artificial perfusion (start of plot) the mean pressure was maintained at 118 mm. Hg by a cardiac output of 1200 cc./min. The heart rate was 160/min. During the first half-hour this mean pressure was approximately maintained; but cardiac output slowly decreased to 1070 cc./min., while the calculated total peripheral resistance (TPR) increased a little.

Approximately at the end of 45 minutes, *B*, the venous pressures started to rise spontaneously, reaching 120 mm. H_2O, but cardiac output was not affected. This passive congestion indicated depression of the myocardium, a feature occasionally noted in other experiments. When no adjustments were made, the heart continued to discharge essentially the same volume under the influence of these greater pressures. Since it was the purpose of this experiment to main-

tain a constant venous pressure, it was necessary to decrease the venous inflow rate. This caused a reduction in cardiac output to 800 < 860/cc. per minute, *C*. An inspection of the graph shows, however, that once reduced to 71 per cent of its control value, cardiac output remained essentially constant for the remainder of the experiment.

With the slight decline of cardiac output during the early period, *A*, *B*, and the greater abrupt reduction at *C*, mean arterial pressure dropped correspondingly. After *D*, a slight recovery to 71 mm. Hg occurred. The pressure pulses —selected samples of which are shown on the lower part of the chart—altered

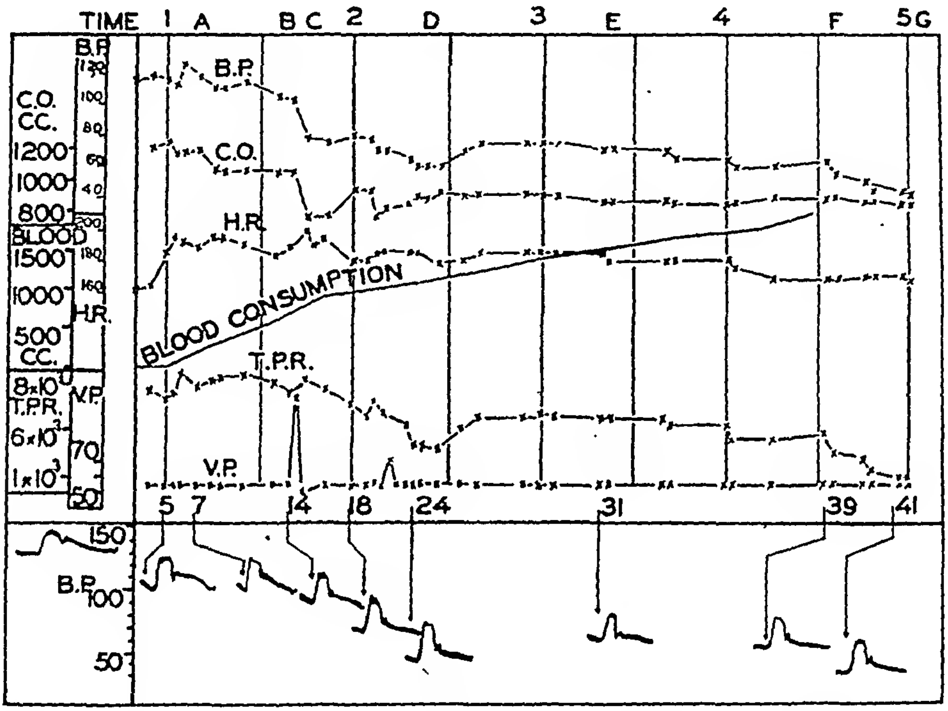

Fig. 2. Plot showing sequential cardio-circulatory changes under conditions described in text. *BP*—mean arterial pressure; *CO*—cardiac output per minute; *HR*—heart rate; *TPR*—total peripheral resistance in absolute units; *VP*—venous perfusion pressure. Below, transcribed aortic pressure pulses showing changes in form and in systolic and diastolic pressure.

in form and decreased in amplitude (curves *5–14*), much as during a large hemorrhage (6). With unchanged TPR, these changes were solely due to decreased systolic discharge. The changes in form and amplitude observed after large hemorrhages may also be due dominantly to reduction in systolic discharge.

While cardiac output remained reduced but constant during the period *D-G*. the heart slowed somewhat and therefore systolic discharge increased a little. The mean pressure nevertheless declined slowly from 71 mm. to 42 mm. during this period. This was solely due to a progressive decline in TPR which became much further reduced at a late stage, *F-G*.

With the early development of declining TPR, the pulse pressure increased

according to rule (curves *14–24*), but as it declined further, pulse pressure became smaller again (curves *31–41*). This illustrates the difficulty of assessing changes in peripheral resistance by pulse pressure variations, probably because a number of variables, such as reflected waves, also affect the pulse amplitude.

In the charts of various figures, the curves labeled "blood consumption" depict the rate at which blood was added to our reservoirs and hence the rate at which it was taken out of circulation by the dog. In the experiment plotted as figure 2 such storage began almost immediately and continued at so fast a rate that at the end of the first hour (2:00 p.m.) a liter of blood had been added to the perfusion system. Subtracting some 250 cc. returned by the animal during the early period of hypotension, the dog had nevertheless added to his own calculated blood volume of 980 cc. another 750 cc., within an hour. During the remainder of the experiment, during which a lower arterial pressure persisted, the rate of blood consumption was somewhat less but progressive, so that at the end the animal had sequestered a total extra blood volume equal to 1900 cc., or almost three times its own blood volume.

Since the quantities thus stored sometimes reached three liters, experiments of this nature offered a unique opportunity for discovering the places in which it is sequestered. The autopsy findings were fairly typical. There was no obvious increase in peritoneal or other serous fluids nor gross evidence of pulmonary congestion or edema. The kidneys, liver and pancreas were not congested and, with two exceptions, the spleen was small and contracted. The leg muscles were dry and not edematous. However, the small intestines from the middle of the duodenal bulb to the lower ileum were swollen and, on opening, the mucosa were always found to be intensely congested and purplish in color. Colored photographs were almost identical with a plate published by Whipple et al. (5) illustrating changes produced by injection of filtered intestinal contents from animals with intestinal obstruction. Hemorrhagic areas and blood in the lumen were not uncommon findings. There seems to be no question that the excess blood was almost wholly stored in the intestinal mucosa, as first reported by Erlanger and Gasser (7) in other forms of shock, and consistently noted by us (6) in hemorrhagic shock. No post mortem evidence of generalized capillary damage in other organs, such as Moon (8) has described, could be found. Only one other pathological change of consequence was noted fairly constantly, viz., superficial subendocardial hemorrhages varying in extent, intensity and distribution. In this experiment they were limited to the right ventricle, in the experiment next analyzed to the left ventricle, and occasionally both chambers were affected. No electrocardiographic changes were associated with these subendocardial hemorrhages. Such hemorrhages were never noted by Werle, Cosby and Wiggers (6) in autopsies on dogs that had died from hemorrhagic shock.

In summary, this, like several other experiments of our series, may be said to simulate experiments on an intact animal in which a fulminating type of shock develops as a result of a progressive decrease in total peripheral resistance, but in which venous pressure remains or is kept constant. The thought suggests

itself that maintenance of a normal venous pressure may not be a satisfactory method for combating such types of shock.

A somewhat similar course of events developed when venous pressure and cardiac output were materially increased to values sufficient to maintain normal or high arterial pressures at the start. Thus, in two experiments, cardiac output volumes of about 1500 cc. maintained mean pressures of 148 and 162 mm. Hg respectively, but arterial pressures fell abruptly during the first half-hour and the animals at once started to consume large volumes of extra blood from the reservoir. Indeed, it soon became obvious that maintenance of large initial cardiac outputs and high initial arterial pressures did not prevent or retard, but rather hastened, the downward trend of arterial pressures and accelerated the consumption of blood.

Shock with maintained or increased cardiac output.

Figure 3 illustrates data from an experiment in which attempts were made to maintain arterial pressures by progressively increasing the venous pressure and cardiac output. The plot of mean arterial pressures, *A*, *B*, illustrates the mode of recovery from a previous six minute period of hypotension (40 > 20 mm. Hg). At the very onset, a venous pressure of 60 mm. and cardiac output of only 630 cc./min. sufficed to maintain mean arterial pressure at 110–120 mm. Hg for about 35 minutes, *A-C*. Shortly thereafter TPR began to decrease rapidly and arterial pressures declined abruptly. The pressure pulses (cf. curves *10*, *11*, *14*) increased in amplitude according to rule, the summit became peaked and the diastolic limb following the incisura was flattened. The blood consumption curve shows that during this preliminary period comparatively little blood had been consumed.

During the observations made at the time indicated by *C* on the plot it was noticed that cardiac output was beginning to decrease a little, hence venous pressure was raised to 67 mm. H_2O to compensate for this reduction. This had little effect on arterial pressures or pressure pulses. At *F*, venous pressure was further raised to 90 mm. H_2O in an effort to maintain arterial pressure by augmenting cardiac output to 1020 cc./min. This represented a 61 per cent increase over the initial output at *A-B*. Through this expedient mean arterial pressure increased to 90 mm. at *E-F*; subsequently fell a little, *F-G*; but toward the end declined rapidly, *G-H*. The pressure pulses remained large and collapsing (curve *33* to *65*).

During the initial decline of arterial pressure (fig. 3, *B-F*), TPR decreased significantly, actually from about 15 × 10^3 to 7.2 × 10^3 A.U.; from *F* to *G* it stabilized and, thereafter, decreased to a final low of about 5 × 10^3 A.U. Attempts to evoke a pressor reaction by stimulation of both vagus nerves with a strong tetanizing current failed.

Attention should be called to the fact that with increase in cardiac output at *F*, the rate at which blood was withdrawn from circulation increased and that this was maintained fairly constant until the end, when 3.2 liters of additional blood or an amount equal to one-fourth of the dog's body weight had been stored.

The autopsy findings again showed no increased blood storage in any other organ than the intestinal mucosa, but this extended to branches of the mesenteric vessels, many of which appeared to be thrombosed.

The time-curve of the mucosal changes was studied during the course of several experiments. In this instance, examination of a duodenal loop temporarily exteriorized at 1:10 p.m. revealed no obvious pathological changes, and a second inspection at 3:05 p.m. of a segment 4 or 5 cm. lower also showed no outstanding congestion, despite the fact that the animal had demobilized nearly 1.5 liters of blood. Apparently the pathological congestive changes at

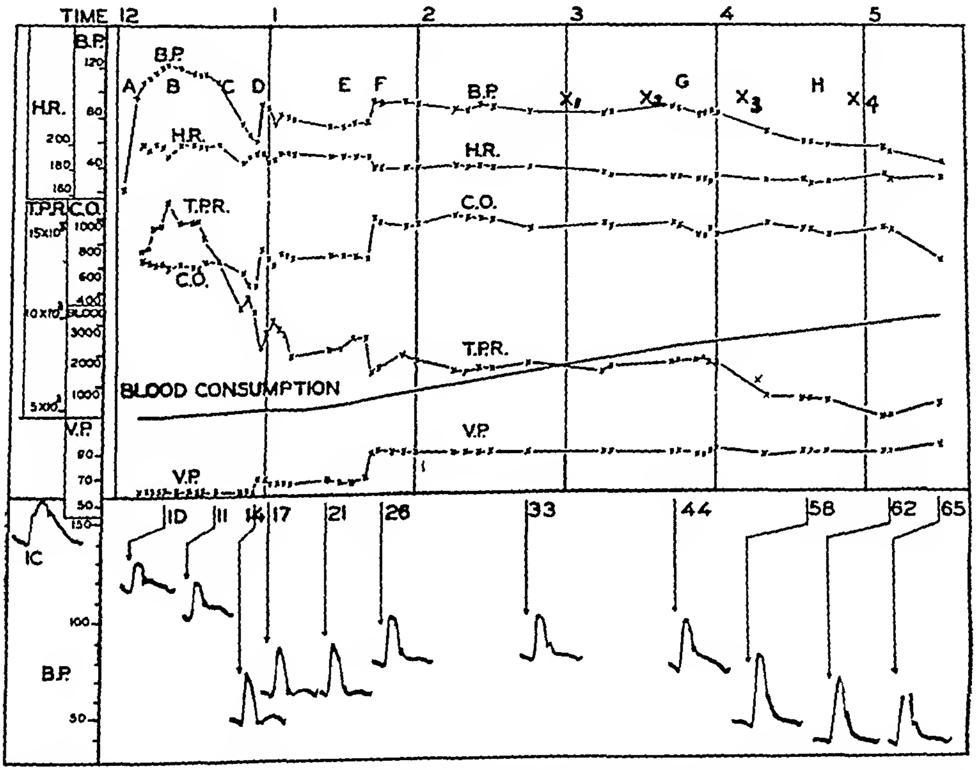

Fig. 3. Plot showing sequence of cardio-circulatory changes under conditions described in text. Designation of curves as in figure 2.

autopsy are not occasioned by a mere mechanical packing of blood into the vessels but seems to require some specific change in blood vessels. This correlates with observations made previously (6) that mucosal changes also occur when there is less blood in the body after prolonged hemorrhagic hypotension. At 4:10 p.m. a third inspection of a segment 4 cm. above the first revealed the characteristic pathological picture. The occurrence of such intensive mucosal changes, despite maintenance of a good cardiac output and arterial pressure, indicates that early deprivation of blood supply, anoxia, etc., is not the only factor concerned with the vascular changes. Since TPR decreased significantly, it could not have been due to vasoconstriction. Maintenance of an arterial

hypotension at 20 mm. for 6 to 8 minutes in an intact dog through hemorrhage also does not produce these changes.

Summarizing this and similar experiments, it is demonstrated that conditions can be created experimentally in which all the dynamic and pathological criteria of shock are produced speedily despite the fact that venous pressure and cardiac output are increased by 50 per cent or more. The causative mechanisms lie wholly in the periphery of the arterial tree. It is also shown that, except toward the end, cardiac output at equivalent venous pressures decreases but little. This was true not only at the pressures plotted, but at temporarily lower venous pressures tested at the intervals marked X^1 to X^4. To prevent confusion these were not indicated on the plot, but are briefly summarized in table 3.

While there is a slight reduction in cardiac output at equivalent venous pressures this proved no greater than may occur in such artificial preparations even though the coronary vessels are fed by a substantial pressure head in the aorta.

TABLE 3

CONTROL AT	VENOUS PRESSURE	CARDIAC OUTPUT PER MIN./CC.	PERCENTAGE OF ORIGINAL OUTPUT
	mm. H_2O		
E-F	90	1020	100
	67	630	100
X^1	90	925–946	92.7
	68	608–613	97.3
X^2	90	919–943	92.4
	68	502–507	90.0
X^3	90	841–891	87.3
	68	507–542	86.0
X^3	90	870–928	91
	70	610–612	97.1

Finally, it should be stated that in the particular experiment chosen as an illustration (fig. 3), the dog had been primed on the previous day with 10 cc. cortical adrenal hormone (Eschatin) and with 20 cc. on the morning of the experiment. In addition, 10 cc. was administered during the artificial perfusion. Since the course of events did not differ from that of untreated dogs, the data from this experiment serve at once to illustrate the natural course of events and to place on record details of one of our negative experiments with adrenal cortical hormone. The results of other experiments are analyzed later in this communication.

Shock with reduced venous pressure and diminished cardiac output. Experiments so far discussed did not duplicate conditions admittedly found in shock, viz., reduction in venous return and secondary decrease in cardiac output. Most of our experiments were naturally designed to simulate such conditions. This could easily be done in our preparation by merely reducing the venous return through lowering of the Mariotte perfusion bottle.

The general procedure and nature of results are illustrated by an experiment

performed on a 13 kilo dog and charted as figure 4. During the control period, *A-B*, a venous pressure of 70 mm. H_2O and a cardiac output of 1055 cc. per minute maintained an arterial pressure of about 120 mm. Hg. After control data had been collected during a 20 minute interval, venous pressures were reduced in two steps, viz., to 50 mm. H_2O at *B* and to 32 mm. H_2O at *C*. This simulated the effects of two large hemorrhages, and in fact an excess volume of about 200

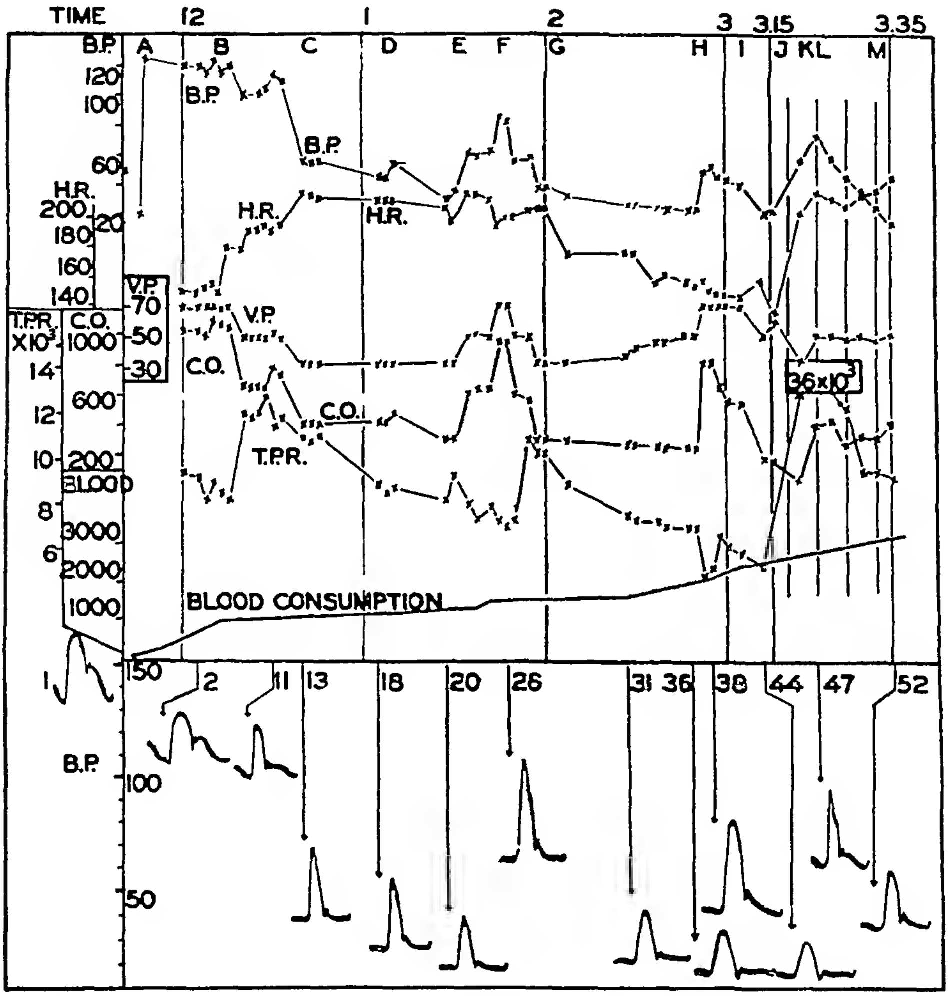

Fig. 4. Plot showing sequence of cardio-circulatory changes under conditions described in text. Designation of curves as in figure 2.

cc. was returned from the animal to the lower reservoir. With the initial decrease in venous pressure and cardiac output at *B*, the heart rate accelerated and TPR increased, as also happens promptly after hemorrhage. With the second reduction at *C*, however, TPR returned to normal and thereafter the progressive trend was distinctly downward.

Selected pressure curves during the early stages (*A-C*) show an increase in amplitude, a peaked and collapsing form and a characteristic flattening of the

diastolic limb (curves *2*, *11*, *18*). Had such curves been obtained in an intact animal, the temptation would have arisen to attribute these phenomena to an initial reduction of TPR. However, in these experiments calculations showed that TPR was higher than the control. Apparently, circumstances can arise during which deductions concerning the circulatory state may not be drawn from pressure pulses on the basis of theoretical deductions or experiments on artificial models.

During the control interval, *A-B*, the net consumption of blood was about 700 cc. After reduction of venous and arterial pressures the blood storage during the period, *C-E*, continued steadily but at a slower rate. Mean arterial pressure slowly declined from 60 to 38 mm. Hg, due almost wholly to a decreasing TPR, and not significantly to reduced cardiac output. At this time it was deemed expedient to determine the cardiac output responses to higher venous pressures (50 and 70 mm. H_2O) used earlier in the experiment. As can be seen at *E*, *F* on the chart, cardiac outputs at these pressures were somewhat less than at *A*, *B*, *C*, but not sufficiently so as to indicate any inordinate depression of the myocardium. It may be pointed out, parenthetically, that with the purposeful

TABLE 4

VENOUS PRESSURE	C.O. AT B-C	C.O. AT E-F	C.O. AT H
mm. H_2O	*cc.*	*cc.*	*cc.*
70	1055	940	685
50	658	620	253
32	406	300	272

two-stage increase in cardiac output *E-F*, mean arterial pressures increased to 80 mm. Hg, but the form of the pressure pulse retained its collapsing feature (curve *26*). Obviously, the rise in mean pressure following an increase in cardiac output does not necessarily signify a degree of improvement ordinarily associated with it.

At *G*, the experiment was continued at low venous pressure. During the 50 minute interval indicated by *G-H*, mean arterial pressure and TPR decreased progressively, cardiac output slowly declined and pressure pulses decreased in amplitude (curves *26*, *31*, *36*). The heart rate now slowed progressively despite constancy of temperature, a sign which seemed to be associated with onset of myocardial failure in hemorrhage experiments reported by Werle, Cosby and Wiggers (6). At *H*, the cardiac output at 32, 50 and 70 mm. H_2O was again tested. Table 4 shows some comparative data at equivalent venous pressures.

Following these tests, *I*, venous pressures could not be reduced below 51 mm. H_2O; even at this pressure cardiac output declined progressively to 170 cc./min. All signs indicated that progressive myocardial depression contributed significantly to the progressive decline of arterial hypotension and that elevation of venous pressure following an increase in venous return did not restore cardiac output.

Analyzing fourteen such experiments, we feel justified in concluding that in

preparations in which a hypotension of 50 mm. Hg or less lasts for 1 to 1.5 hours, depression of the myocardium occurs, in the sense that its capacity to eject equal blood volumes at equivalent venous pressures is decidedly reducd.

The effects of neosynephrine at terminal stages. During the terminal stages of five experiments we tested the cardiac and vascular responses to neosynephrine and their effect on the course of events. Tests earlier in the course of our experiments appeared inadvisable; they might have obscured the natural course of events. Neosynephrine was chosen, because the pressor effects are more sustained than those of epinephrine and other pressor amines of the catechol group, and the doses necessary to produce a given pressure response is less than for other members of the phenol group of pressor amines. Furthermore, according to Crismon and Tainter (9), its pressor potency is greater in circulatory depression following destruction of the brain and medulla than it is in normal animals.

A typical reaction following addition of 0.25 cc. of a 1 per cent solution of neosynephrine[6] to the lower blood reservoir is shown in figure 4 from *J* to *M*. Mean pressure increased rapidly from 20 to 70 mm., the slowed heart accelerated and TPR increased so tremendously that it is indicated numerically (36×10^3 A.U.). Such reactions certainly proved that the capacity of peripheral vasoconstriction has not been lost in our preparation. The blood pressure reactions resembled those obtained on animals after section of the spinal cord or destruction of medullary centers (9). The venous pressure initially fell, *J*, *K*, indicating a temporary increase in cardiac output between flowmeter readings; but, as the venous pressure decreased, the metered cardiac output at *K* was actually less. Thereupon, we increased venous inflow sufficiently to restore venous pressure to the previous level, *L*. As a result, cardiac output augmented. Comparison of pressure pulses 44 and 47 show, however, that a natural form was not restored as arterial pressure rose.

During the following 15 minutes, *L-M*, the vascular effects gradually waned; TPR dropped but remained above previous levels, as did the blood pressure which had a terminal value of 40 mm. Hg. The after-effects on the heart, possibly due to temporary improvement in its blood supply were more persistent. The heart remained rapid and cardiac output did not decline. The final pressure pulse (curve *52*) shows a definite improvement when compared to that of curve *44* taken previous to use of the drug.

The effects of cortical adrenal hormones. When a sufficient number of experiments had been performed to convince us that the dynamic and pathological intestinal changes could not be prevented by experimental expedients, it occurred to us that this preparation offered a supreme opportunity to test the value of cortical adrenal hormones. If the adrenal hormones could prevent or retard the fulminating circulatory collapse and avert or ameliorate the damage to the intestinal mucosa, their value would be demonstrated beyond all doubt; but we, of course, recognized from the start that failure to achieve such results would

[6] We are indebted to Frederick Stearns and Co., Detroit, for the neosynephrine utilized in these experiments.

not necessarily establish their inefficiency in less violent and more slowly progressing forms of shock.

Three dogs were treated with the commercial preparation, Eschatin,[7] containing 25 dog units per cubic centimeter, and two dogs with a preparation of whole adrenal cortex (702)[7] representing 50 grams adrenal cortex per cubic centimeter. The dogs received a large priming dose early in the morning before the experiment, and repeated additions were made to the blood reservoirs during the experiment. Two dogs received an additional intramuscular priming dose the afternoon before an experiment. Table 5 shows that the total doses given are many times those necessary to support an adrenalectomized animal.

A survey of our results, either as regards the course of dynamic events or the pathological findings in the intestinal mucosa, fails to disclose evidence of any beneficial influence. One of the experiments has already been described in detail.

Discussion. *The rôle of myocardial depression.* Suspicions that some degree of myocardial depression occurs during shock have been voiced by a number of

TABLE 5

EXP.	PRIMING DOSES (INTRAMUSCULAR)	TOTAL DOSE	PREPARATION
	cc.	*cc.*	
1	5	10	Eschatin
2	2.5	8.5	Eschatin
3	3.5	20	Kendall preparation
4	10*	20	Kendall preparation
5	10*	40	Eschatin

* 5 cc., previous afternoon; 5 cc., early morning of experiment.

investigators, among them Howell (10), Y. Henderson (11) and Erlanger and Gasser (12). In recent reports from this laboratory (1, 6) it was suggested that its importance had perhaps been minimized in recent years. However, the question whether it *a*, is concerned in the initial reduction of cardiac output and initiation of shock; *b*, acts solely as a contributing factor; *c*, is a factor in development of an irreversible circulatory state, or *d*, causes the terminal circulatory failure, remained in a speculative stage.

In the type of experiment here reported, the myocardial depression which follows prolonged extreme hypotension is a comparatively late phenomenon which appears definitely after the process of blood sequestration is well under way and the intestinal mucosa has become extensively congestive and hemorrhagic.[8] In comparison with intestinal vessels, the myocardium and central

[7] We are indebted to Parke Davis and Co. for the supply of Eschatin, and to Dr. E. C. Kendall for the preparation no. 702 used in these experiments.

[8] Obviously, this deduction is predicated on the supposition that the initial depression of the myocardium which apparently accompanies the transfer to an artificial perfusion system (p. 215) is not a part of the shock picture. This could be argued, but we have adopted the view which is least favorable to a hypothesis that myocardial failure is of importance during the development of shock.

nervous system show a remarkable degree of resistance to shock-producing agents. Myocardial depression is therefore not an initiating factor. Irreversibility also is established before myocardial depression sets in.[8] However, there is no question that when peripheral processes have advanced considerably, myocardial depression contributes to the suddenness of the circulatory failure, and when failure of the respiration does not occur previously it is responsible for the terminal circulatory failure.

The significance of reduced total peripheral resistance. In previous studies Wiggers and Werle (1) noted that, during and after development of hemorrhagic shock, the calculated TPR decreased to or below control values. Since the state of vascular resistance at the start of observations remained unknown and the magnitude of changes required to make a diagnosis of reduction in TPR had not been established, we made the more conservative statement that TPR is not increased in every form of shock, as is currently believed.

In experiments now reported, there can be no doubt that the significant and constant changes during our observation periods consisted in a steady and pronounced decrease in TPR. Whether this was of central or peripheral origin we are not sure.

Such results do not contradict the continuance of vasoconstriction in structures of the limbs, for as one of us (13) has analyzed, changes in splanchnic resistance apparently dominate total peripheral resistance. Nor can these observations necessarily be interpreted as opposed to a generally accepted view that generalized vasoconstriction is the dominant characteristic during early stages of shock. As previously pointed out (4), many contradictory interpretations regarding the mechanism of shock which occur in the literature have been due to the fact that differences in experimentation or in the condition of the animal at the start were not always scrutinized carefully. We must guard against such pitfalls to the best of our ability.

Again reviewing our results, it should be emphasized that in some of our experiments, as illustrated by that of figure 3, TPR increased initially. In that larger number of experiments in which a decrease in TPR developed progressively from the start it could be assumed that the phase of intensive generalized constriction occurred previous to our observations and, further, that shock-inducing processes had started to work during the operative procedures which preceded. The apparent existence of an increased TPR at the start of our experiments harmonizes with such a view. Since most experimenters agree that the preliminary phase of intense vasoconstriction during shock is followed by a phase of reduced peripheral resistance, it is possible that in our experiments the phase of increasing TPR was obscured and the later phase of decreasing TPR was made especially prominent.

Consequently, while we cannot be certain from our experiments whether increased or decreased TPR were initiating factors, it seems clear that development of an irreversible state correlates with a reduction in TPR.

Applicability of results. It is obviously necessary to apply our results and interpretations to other forms of shock with the greatest care. It is conceivable that the clear-cut results obtained in our preparation may have started us on a

false lead, as regards general applicability or, indeed, applicability to any form of shock. It may again be pointed out, however, that contrary deductions regarding the state of peripheral resistance when true shock has begun to develop are not based on rigid application of experimental data (cf. Wiggers, 4). No one has yet succeeded in evaluating or following changes in TPR in intact shocked animals. Only when contrary proof has been adduced by such studies can it be stated with certainty that reduction in TPR is not the factor which determines irreversibility, without which a state of true shock can probably not be recognized. For ourselves, we are prepared to change our viewpoint repeatedly, meanwhile recording experimental facts from time to time regardless of whether they will eventually prove to have or not to have a practical bearing.

In conclusion, we would re-emphasize that while reserve and caution must be exercised in applying the results of these experiments to forms of shock which develop more slowly, they may have a bearing on more fulminant types, not entirely unknown clinically. Every victim of a serious accident, not immediately killed, who is transported to an emergency station *in extremis* and expires within a few hours despite routine efforts to save his life must be suspected of having died from a fulminant type of shock. This is but one of many clinical examples which suggest themselves. Too little attention has perhaps been given both to the diagnosis of fulminant shock by clinicians and to its study and analysis by investigators. The possibility that fulminant shock arises as a result of a rapid development of the phase of reduced total peripheral resistance and may require a different routine management must also be considered.

SUMMARY

1. The relative importance of *a*, decrease in venous pressure and secondary reduction of cardiac output; *b*, primary myocardial impairment, and *c*, changes in total peripheral resistance (TPR) in shock was studied by means of a heart-lung-dog preparation, which is described. Venous flow and cardiac output were under control and TPR could be calculated. In such a preparation, a fulminant type of shock developed spontaneously which may resemble clinical types characterized by speedy circulatory failure and death.

2. In twenty-four such experiments it was shown *a*, that shock can develop without progressive reduction or even with a rise of venous pressures or cardiac output, and *b*, that it cannot be prevented or cured by increasing venous inflow and cardiac output.

3. The fundamental factor responsible for this type of irreversible circulatory failure was a steady and pronounced decrease in total peripheral resistance, which enabled the animal to store, over and above its own blood volume, quantities of blood equal to 25 per cent of its body weight or four times its own blood volume. Autopsies revealed no storage depots other than the mucosa of the upper intestines which was always edematous, intensely congested and often hemorrhagic. The integration of these observations with apparently contrary findings regarding the state of the peripheral circulation, and their application to fulminant types of shock in man are discussed.

4. Following any prolonged period of hypotension during the development of shock, the cardiac output at equivalent venous pressures also decreased, indicating that depression of the myocardium occurred. In the form of shock studied, this did not prove to be an initiating factor nor was it necessary to produce an irreversible state. But, after the peripheral changes of shock had been well established, it played an important rôle in the rapid downward trend of blood pressure and was the ultimate cause of death.

5. Administration of neosynephrine during terminal stages evoked a tremendous increase in peripheral resistance and increased cardiac output at equivalent venous pressures, indicating that the power of vascular constriction was not lost and that the myocardium was stimulated. While this resulted in a marked increase in mean arterial pressure and apparent improvement lasting over half an hour, the optical pressure pulses had a peaked summit and collapsing character, proving that dynamic improvement cannot be judged by elevation of mean pressure alone.

6. Five dogs primed with liberal doses of cortical adrenal extracts and receiving these extracts during the experiments, revealed no indications that the course of dynamic events or the pathological changes in the intestinal mucosa were influenced. It must be recognized, however, that our preparations perhaps offered too severe a test.

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INCREASED ERYTHROCYTE DESTRUCTION ON A HIGH FAT DIET[1]

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Lymph samples collected from the lacteals and thoracic ducts of dogs following fat ingestion and absorption are markedly hemolytic; over half of the erythrocytes in samples of heparinized blood may be destroyed by mixing the blood with fatty chyle (Johnson and Freeman, 1938). The hemolytic factor in chyle is probably soap plus free fatty acids, which are present in chyle during the period of rapid fat absorption in quantities (ranging from 3.3 to 6.3 mgm. per cubic centimeter) sufficient to account for the hemolytic action of chyle (Freeman and Johnson, 1940).

Since this hemolytic action is demonstrable in chyle-blood mixtures in vitro, the question arises whether hemolysis actually occurs in the blood stream following fat ingestion. The work reported here is an attempt to determine whether a high fat diet accelerates red blood cell destruction. Following fat ingestion and absorption, chyle reaches the blood stream by way of the thoracic duct and other routes (recently reinvestigated by Freeman, 1942), and the circulating blood is exposed to a substance which has been demonstrated to be extensively hemolytic in vitro. When red blood cells are destroyed in the blood stream, the porphyrin constituents of the split hemoglobin are quantitatively excreted in the bile of dogs as bilirubin (Broun, McMaster and Rous, 1923; Hawkins and Johnson, 1939). Hence, the determination of the total bilirubin output provides a reliable quantitative measure of the rate of red blood cell destruction, even in the presence of normally functioning hematopoietic tissue.

METHODS. Internal bile-fistula dogs were prepared both in the manner described by Kapsinow, Engle and Harvey (1935) and by anastomosing the common bile duct with the right ureter (after removing the gall bladder and the right kidney), so that the total bile output was diverted to the urinary bladder and excreted with the urine. The dogs were placed in metabolism cages and daily collections of the 24-hour urine-bile mixtures were begun when the animals were in good clinical condition and gaining weight.

The daily 24-hour samples were filtered through gauze and the volume measured. Bilirubin determinations were made daily by a modification of the oxidative process of Malloy and Evelyn (1937). An aliquot portion of the urine-bile mixture was diluted with ethyl alcohol (95 per cent) to which was added the oxidizing agent, consisting of hydrogen peroxide in acid alcohol (0.5 cc. of 30 per cent H_2O_2 in 94.5 cc. of 95 per cent ethyl alcohol plus 5.0 cc. of concentrated HCl). The aliquot was usually 2.0 cc., since this quantity most often resulted

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

in a reading which fell in the center of the galvanometric scale of the Evelyn photoelectric colorimeter. When samples were highly concentrated, accurate dilutions were made to give readings in the proper range. Readings were made on all samples every one-half hour until a maximum blue color was developed. By this method quantitative recovery of known amounts of bilirubin (Eastman Kodak Company) added to urine was possible with an error not in excess of 2.0 per cent.

In addition, external bile-fistula dogs were prepared and bile was collected under constant negative pressure in the manner described by Kocour and Ivy (1938). Bile samples were collected every 24 hours and bilirubin determinations were carried out (using 0.5 cc. aliquot portions) as described above.

All dogs were maintained on a basal diet consisting of canned commercial dog food supplemented daily with cod liver oil (4.0 cc.), yeast (0.5 gram), iron (ferrous ammonium citrate, 100 mgm.), and bone meal (sufficient to control stool consistency). Each dog also received a daily dose of 0.5 gram of pure sodium taurocholate per os before feedings. Water was given ad libitum. To this basal diet was added olive oil or corn oil in amounts of from 5.0 to 10.0 cc. per kilogram of body weight to comprise the "high fat" diet. Cane sugar was substituted for the olive or corn oil in calorically equivalent amounts to comprise the "high carbohydrate" diet. Observations were continued for several months on most dogs. For each animal the diets to be compared were alternated several times. Each dietary regimen was usually maintained for a period of eight days to three weeks.

RESULTS. The results of adding fat to the basal diet are shown graphically in figure 1. The average daily total output of bile pigment in milligrams is plotted against the number of days of observation. The figure indicates that at each shift from the control basal diet (cross-hatched) to the high fat diet (solid) the bile pigment output increases. With each change from high fat to control, the output decreases. The daily fluctuations in bile pigment excretion (not shown in fig. 1) are considerable. However, statistical analysis of the data on daily excretions for the 133 days of the experiment shows the bile pigment output to be significantly higher on the high fat diet.

To determine whether this increase might be caused by the increased caloric intake on the high fat diet, observations were made comparing bile pigment excretion on the high fat diet and on a diet in which this fat was replaced by calorically equivalent quantities of cane sugar. The results are plotted in figure 2, which shows that bile pigment excretion is greater on a high fat diet than on a calorically equivalent high carbohydrate diet. Statistical analysis of the figures on daily excretion for the 144 days of the experiment shows the differences to be significant. Hence, it appears that the increase in bile pigment output which occurs when dogs are placed on a high fat diet is due not to an increased caloric intake, but to increased red blood cell destruction produced by the products of fat digestion.

The composite results of observations on seven dogs are shown in figure 3. Here the average daily output of bile pigment for all dogs during all control

periods (cross-hatched column) is arbitrarily placed at 100 per cent. Each of the 24 points in the control column represents the average daily excretion of about 21 days of observations, totalling 511 dog-days. The average output during

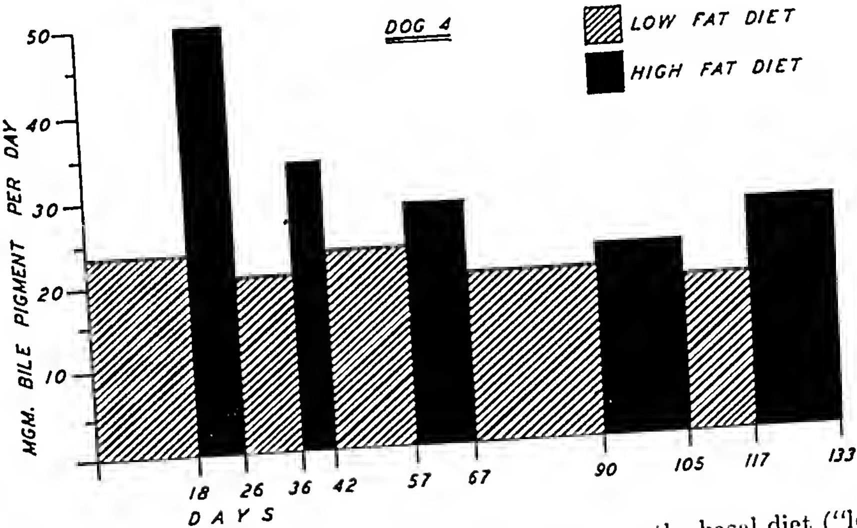

Fig. 1. Average daily excretion of bile pigment by a dog on the basal diet ("low fat"), and on the basal diet plus fat ("high fat").

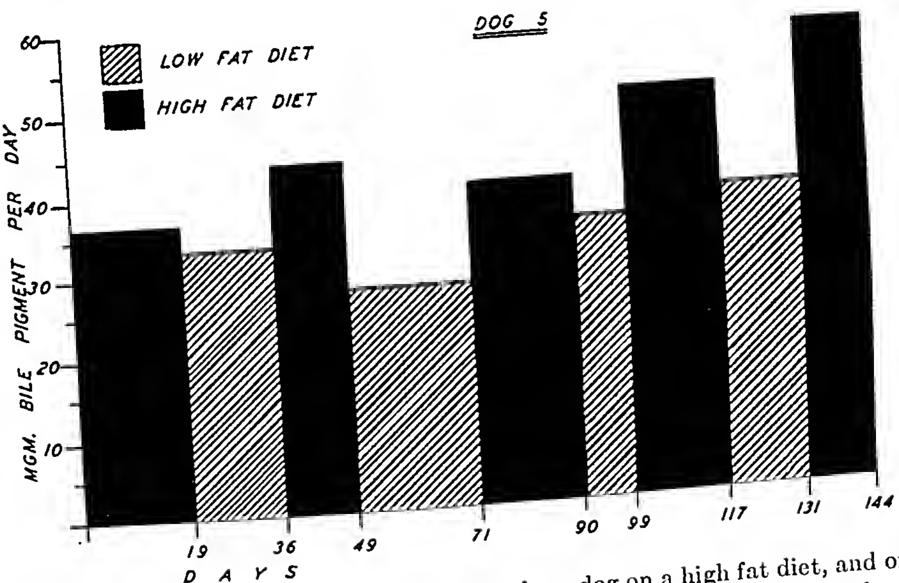

Fig. 2. Average daily excretion of bile pigment by a dog on a high fat diet, and on a low fat diet in which the fat was replaced with a calorically equivalent quantity of carbohydrate.

the administration of the high fat diet (solid column) is 137 per cent. Each of the 20 points in this column represents the average of about 16 days, the total being 325 dog-days. Statistical analysis reveals that the difference is significant despite the fact that two dogs responded poorly to the changes in diet.

Similar results were obtained following dietary changes in two external bile-fistula dogs.

Routine red blood cell counts made at weekly intervals in all of the experimental animals varied from week to week, but did not correlate with fluctuations in bilirubin output. No significant anemia was detected in any of the animals at any time.

Discussion. It has been postulated that red blood cells are destroyed in the body by phagocytosis, hemolysis and fragmentation. Rous (1923), in reviewing the literature, concludes that there is no positive evidence supporting any of these mechanisms or any combination of them. Isaacs (1937) points out that there is little evidence for phagocytic destruction. He states that a hemolytic mechanism is logical but has not been demonstrated. Fragmentation of red blood cells has been observed in peripheral blood in erythroblastic anemia

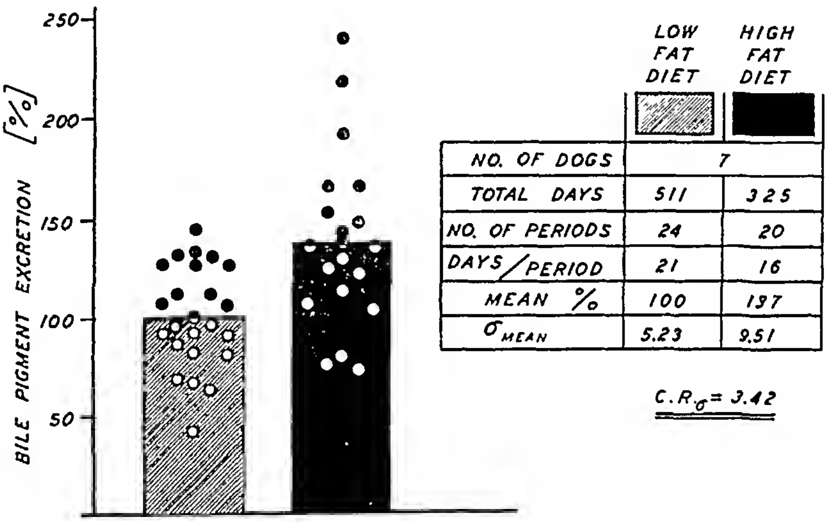

Fig. 3. Summary of results on bile pigment excretion in seven dogs on high fat and low fat diets.

(Cooley and Lee, 1933), and in other pathological conditions (Doan and Sabin, 1926). From the present work it would appear that the red cell destruction by chyle is probably by hemolysis, and that this mechanism may play a rôle in the normal red blood cell destruction in dogs. Further support is lent this concept by the experiments of Longini, Freeman and Johnson (1942), demonstrating that red blood cells exposed to lipemic serum are more fragile than cells exposed to fasting serum. Less direct evidence of red blood cell destruction from fat ingestion, based on studies of urobilin excretion, has been presented in a review by Glanzman (1929) and in a preliminary report of experiments by Josephs, Holt, Tidwell, and Kajdi (1938).

Hawkins and Whipple (1938) estimated the average life of the red blood cell at 124 days in dogs by determination of the time interval between the immediate rise in bile pigment output following massive destruction of circulating red cell and a later massive rise in bile pigment output. Calculating the average life of

red blood cells from the average daily output of bilirubin in the present studies yields the figure of about 200 days.

One might expect that, since a high fat diet causes increased red blood cell destruction in dogs, prolonged fat feeding would result in an anemia. In the present series of animals the number of erythrocytes remained essentially constant even though they were destroyed at an increased rate. The normally functioning hematopoietic tissue seemed able to compensate for the extra losses induced by fat feeding.

A blood picture simulating pernicious anemia has been reported by Crandall, Finne and Smith (1941) in bile fistula dogs somewhat like our own. However, this finding could scarcely be attributed to fat ingestion, since bile salts were withheld from the diet of their dogs, and presumably fat absorption was minimal.

Sodium taurocholate (Merck-"Pure") was fed our dogs in amounts calculated to substitute for the normal daily secretion. Pure bile salts have been shown not to influence bile pigment output (Hooper, 1916; Berman, Snapp, Atkinson, Ivy and Hough, 1940). Whole bile was fed at no time, as it was felt that absorption and re-excretion of bilirubin (Broun, McMaster and Rous, 1923) might affect the results. Although the stools were consistently clay-colored, they were not fatty.

Whipple and Hooper (1916) have reported an increased bilirubin output in dogs placed on a high carbohydrate diet. Rous, Broun and McMaster (1923) reported that no variation occurred in 24-hour samples from feeding a high carbohydrate diet except for a transient hastening of bilirubin output for a few hours. The latter workers suggested that the six-hour collections of Whipple and Hooper were inadequate. In the present series it was felt that possible caloric effects could be eliminated by feeding calorically equal carbohydrate control and experimental fat diets. Feeding periods were usually maintained for eight or more days, since it was observed in preliminary experiments that the response to a change of diet failed to occur, in some instances, until two or three days had elapsed.

Other factors which have been demonstrated to influence bilirubin output in bile-fistula dogs are operative trauma, exercise, infection, fever, and intercurrent diseases (McMaster, Broun and Rous, 1923; Broun, 1923). These factors were adequately controlled in the present work. After returning to good clinical condition the animals showed no evidence of fever or infection, daily exercise was only moderate, and body weight was maintained or increased. One dog died after 24 months; five were sacrificed after 21 months. Autopsies revealed only a left kidney infarct in one animal.

SUMMARY AND CONCLUSIONS

1. In five internal bile-fistula dogs the daily total bilirubin output was significantly higher on high fat diets than on calorically equivalent low fat diets.

2. This effect was also demonstrated in two external bile-fistula dogs.

3. These findings indicate that red blood cell destruction proceeds at a faster rate on a high fat diet than on a low fat diet; this effect is probably caused by the

introduction of a hemolytic substance into the blood stream by way of the lymphatics.

4. These sequelae of the ingestion of fat are probably one mechanism for normal red blood cell destruction in dogs.

5. Calculations from the average daily bilirubin output in this series indicate that the average life of the red blood cell in dogs approaches 200 days.

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THE SECRETION OF ALKALINE PHOSPHATASE BY THE DOG'S INTESTINE

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The presence of alkaline phosphatase in the small intestine has been recognized for many years. Grosser and Husler (1) and Levene and Medigreceau (2) were among the first to determine the activity of this enzyme in the intestinal mucosa, and since then extracts of the mucosa have been used as a potent source of the enzyme. In his review, Kay (3) points out that the mucosa of the small intestine had the highest phosphatase activity of all the animal tissues studied.

However, little attention has been paid to the phosphatase activity of the intestinal secretions. Levine and Dillon (4) prepared a nucleotidase from the secretions collected from an intestinal fistula in the dog. In his monograph, Robison (5) states that phosphatase is secreted by the small intestine without adducing any experimental evidence for its secretion, assuming perhaps that since it is present in such high concentrations in the mucosa it would appear in the succus entericus. In a recent review of the intestinal secretions, Florey et al. (6) refer briefly to Levene's nucleotidase but do not classify phosphatase as an enzyme regularly present in the intestinal juices.

The present investigations were undertaken in order to determine if phosphatase is a constant and active component of the intestinal secretions and to ascertain any factors that might influence its activity.

Materials and methods. Two groups of experiments were carried out. In one group an enterostomy was performed on dogs at three levels: the upper jejunum, the lower jejunum and the lower ileum. A small rubber tube, through which samples of the intestinal contents could be collected, was inserted at each of these levels and exteriorized through a stab wound. Four dogs were prepared in this manner and the effects of various drugs and diets on the phosphatase activity of their intestinal secretions were determined.

The animals were fasted for twenty-four hours and then were fed a test meal either ad libitum or by stomach tube, depending upon the nature of the meal. Two hours after feeding the animals were placed in stocks and samples of their chyme were collected for a period of two hours. Immediately upon collection the samplings were centrifuged for a half-hour at 2200 r.p.m. The phosphatase activity and inorganic phosphorus of the supernatant portion were determined. Phosphatase activity was measured by the method of Bodansky (7) and inorganic phosphorus by the method of Kutner and Lichtenstein as modified by Bodansky (8). The test meals were meat, milk, casein, olive oil, corn oil, and sucrose.

Another series of experiments was carried out in which the animals were injected with 3 to 5 mgm. of eserine subcutaneously following the period of fasting.

Collections of the intestinal contents and phosphatase and inorganic phosphorus determinations were carried out in the manner outlined above.

In a second group of experiments, isolated loops, 12 inches long, of the duodenum, jejunum and ileum were prepared in dogs under nembutal anesthesia. The loops were thoroughly washed with saline, after which the proximal end was closed off and a cannula inserted in the distal end. The animals were injected with 3 to 5 mgm. of eserine subcutaneously and the secretions of each loop collected hourly for a period of three hours. The collections were centrifuged immediately and phosphatase and inorganic phosphorus determined in the manner described above.

Some observations were also made on the secretions collected from jejunal and colonic fistulae of unanesthetized dogs.

RESULTS. *The influence of diet on the phosphatase activity of intestinal chyme.* A high phosphatase activity was found in the intestinal samplings following all

TABLE 1

DIET	DUODENUM		JEJUNUM		ILEUM	
	Phosphatase*	Inorganic P†	Phosphatase*	Inorganic P†	Phosphatase*	Inorganic P†
Casein (7)	2170	62.9	4404	78.0	23989	107.3
Olive oil (10)	2475	6.5	6830	4.2	19799	3.4
Corn oil (3)	2030		10512		21813	
Meat (11)	1034	89.5	4278	55.9	11862	18.7
Milk (8)	725	54.4	2966	32.4	5168	12.6
Sucrose (8)	842	9.6	1058	4.8	6358	7.2
Eserine (5)	2166	4.3	5168	8.7	9930	10.0

Bracketed numbers indicate the number of experiments.
* Bodansky units per 100 cc. of intestinal contents (average).
† Milligrams per 100 cc. of intestinal contents (average).

the test meals and eserine injection. These results are shown in table 1. The greatest activity at each of the three levels of the intestine was obtained after the casein and oil feedings. The average values in Bodansky units after casein were 2170, 4404, and 23989 and after oil were 2252, 8671, and 20806 at the duodenal, jejunal and ileal levels respectively. The activity of the chyme was about half as great following the meat meal and the least activity was obtained following the milk and sugar feedings.

There is an increase in the phosphatase activity of the intestinal contents as they pass caudalward, the values of the ileal chyme being 7 to 10 times greater than those of the duodenum.

The secretion of phosphatase by isolated intestinal loops. The phosphatase activity of the secretions collected from the duodenal, jejunal and ileal loops is shown in table 2. The duodenal and jejunal secretions exhibited the greatest activity, 2491 and 2048 Bodansky units, respectively. The phosphatase values of the ileal secretions were definitely lower, 429 units. The activity gradient

of the isolated loops is thus the reverse of the gradient found in the experiments on the enterostomized dogs described above. This would indicate that the increased activity of the ileal chyme collected from the enterostomized dogs is probably due to concentration of the intestinal contents as they pass down the intestinal tract and does not represent a greater secretion of the enzyme by the lower part of the gut.

The activity of the hourly collections remained fairly constant over the total collection period of three hours. The volume of the secretions from the duodenum and jejunum averaged 15 to 20 cc. an hour and the ileal flow, 3 to 5 cc. an hour.

A high phosphatase activity was also found in the secretions obtained from dogs with chronic jejunal Thiry-Vella fistulae. These values agree with those found in the jejunal secretions of the acute dogs. The secretions of the chronic colon fistulae exhibited the least activity, 250 Bodansky units per 100 cc. of secretion. These results are shown in table 2.

TABLE 2

LOCATION OF LOOP	PHOSPHATASE*	INORGANIC P†
Duodenum (12)	2491	5.3
Jejunum (12)	2048	3.8
Ileum (12)	429	2.9
Chronic Thiry-Vella loop of jejunum (14)	2118	3.3
Chronic Thiry-Vella loop of colon (7)	252	1.2

Bracketed numbers indicate number of experiment.
* Bodansky units per 100 cc. secretion (average).
† Milligrams per 100 cc. secretion (average).

Discussion. The observations on the enterostomized dogs indicate that there is a high phosphatase activity of the intestinal contents at all times. Since the samplings represented the mixed secretions of the stomach, small intestine, pancreas, and bile, the phosphatase activity of each of these secretions must be considered in determining the major source of the enzyme in the intestinal fluids. The phosphatase activity of gastric and pancreatic juice is negligible. Bile, however, does contain considerable quantities of the enzyme. According to Freeman and Ivy (9) fistula bile of the dog has an activity of 35 to 118 Bodansky units per 100 cc. and gall bladder bile may have values several times higher. Armstrong et al. (10) found higher values for liver and bladder bile, using a different substrate and unit of activity, however. These latter workers have also shown that the fecal phosphatase of bile fistula and obstructed bile duct dogs remains at its typically high level despite the exclusion of bile from the intestinal tract.

Since the phosphatase activity of the intestinal contents at the duodenal level is greater than the reported values for bile and since the bile must undergo dilution by the other secretions and the ingested food, it can be assumed that the

greater part of the phosphatase activity of the chyme is due to the secretions of the intestinal mucosa. Similarly, the great activity of the enzyme in feces most probably has its source in the intestinal secretions.

The high phosphatase activity of the uncontaminated secretions of the isolated intestinal loops in both the acute and chronic dogs lends support to these assumptions.

Practically all collections of succus entericus are contaminated by cellular debris and this fact has led some investigators (Florey et al., 6) to question the reported presence of various enzymes in the intestinal juices as due to a true secretory activity of the mucosa. Whatever the merits of such a criticism may be, the great phosphatase activities found in all the samples of the intestinal secretions, collected under various conditions and subjected to an immediate and vigorous centrifuging, could hardly be accounted for by cell contamination alone. Nor did the activity of samples before and after centrifuging show any change in several instances in which such estimations were carried out. The experimental evidence presented indicates that alkaline phosphatase is secreted by the intestinal tract in varying but considerable quantities, the duodenum and jejunum being the major sites of its secretion.

The significance of the increased enzyme activity of the chyme following the high fat and protein meals is not clear. The question of the adaptive secretion of the small intestine is a confused and controversial one. Bourne et al. (11) have shown that the secretions of an isolated intestinal loop in the dog are richest in enzymes following high fat and protein diets and least after carbohydrate, although there was no change in the relative concentrations of the enzymes studied. Since in the present investigations no observations were made on the activity of other enzymes, it cannot be stated whether the increased phosphatase activity after fat and protein feedings is a specific response or not. Cera and Bellini (12) have reported an increase in the phosphatase activity of the intestinal mucosa of the albino rat during fat absorption. Westenbrink (13), however, found no discernible relationship between the character of the diet and the phosphatase activity of intestinal mucosa in the rat.

The choleretic action of protein and fat and the cholagogic action of fat may also contribute to the increased activity following these meals by augmenting the amount of bile in the intestinal tract.

The function of alkaline phosphatase in the intestinal tract still remains to be ascertained although the wide occurrence of monophosphoric esters in food would suggest a plausible rôle for the enzyme in the digestive process.

SUMMARY

1. The phosphatase activity of duodenal, jejunal and ileal contents obtained from enterostomized dogs was determined following various test meals.

2. The greatest activity was found after protein and fat meals and the least following carbohydrate.

3. The secretions of isolated intestinal loops of dogs with acute or chronic

Thiry-Vella fistulae contain large quantities of phosphatase. The degrees of activity of various segments of the intestinal tract are as follows: duodenum and jejunum > ileum > colon.

4. Phosphatase is considered to be a true secretion of the intestinal tract; its function and the effect of diet upon its activity are discussed.

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INFLUENCE OF NEPHRECTOMY ON OVARIAN RESPONSE TO GONADOTROPINS

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It is possible, since attempts to demonstrate differences in chemical structure for the various gonadotropins have been unsuccessful (1), that the prosthetic groups[1] of all the gonadotropins partake of the same general structure, any difference in physiologic response being the result of differences in physical properties of the molecule or micelle as a whole, which might affect the rate of resorption, permeability, destruction, excretion or stimulation to anti-body formation. The importance of the rate of resorption has been established by investigation on divided dosage (2), on administration of insoluble combinations (3), and on retarding or accelerating body fluid exchange (4). It is the purpose of this paper to extend the study to the kidney to determine whether or not this organ is involved. To this end a comparison has been made between intact and partially nephrectomized rats of the action of three characteristic gonadotropins from sheep pituitary, pregnant-mare serum, and urine of human pregnancy respectively.

Parkes and White showed that 30 per cent of injected prolan appeared in the urine of the rabbit within 9 hours (5). This observation was confirmed by Lipschütz (6), who found in addition that prolan disappeared less rapidly from the blood of the nephrectomized rabbit than it did in the normal rabbit. Evans, Simpson and Austin (7) found prolan in the urine of the rat injected with this substance. These workers and Catchpole, Cole and Pearson (8) were unable to recover mare-serum hormone from the urine of injected animals. Data on the excretion of pituitary hormone by the rat kidney are not available, so that an experiment to cover this point was included.

EXPERIMENTAL PROCEDURE AND RESULTS. *Excretion of sheep pituitary hormone by the normal rat.* The urethras of five 200 to 300 gram female rats were tied off under ether anesthesia. Eight to 11 mgm. of sheep pituitary gonadotropin in saline solution was injected subcutaneously. The bladder contents were collected 6 hours later. The urine was treated with an excess of tannic acid. The precipitate which formed was removed by centrifugation and taken up in a volume of isotonic saline solution corresponding to the volume of urine used. The tannic acid suspension was injected into 22-day-old female rats in 4 divisions once daily in an amount which would correspond to 1.7 to 3.0 mgm. of original hormone if all appeared in the urine unchanged. Tannate suspensions

[1] It is of course also possible that prosthetic groups, in terms of specific atomic linkages are non-existant in the protein molecule, physiologic activity depending upon specific conditions for adsorption (secondary valence effects).

of the original hormone were administered to 22-day-old female rats at 3 dosage levels.

	DOSE PER RAT	AVERAGE WT. OF OVARIES	AVERAGE WT. OF UTERUS	NO. OF RATS
	mgm.	*mgm.*	*mgm.*	
Original hormone	1.0	63		6
	0.5	34	89	6
	0.2	12	73	6
	0.0	14	17	4
Urine precipitate equivalent to original hormone if all were excreted	1.8	14	29	4
	1.7	14	15	3
	3.0	14	15	3

The experiments establish that less than 10 per cent of the hormone was present in the urine in unchanged form 6 hours after administration.

Administration of three gonadotropins to nephrectomized rats. Twenty-two-day-old female rats were partially nephrectomized by the technic of Chanutin and Ferris (9). The first dose of hormone was administered within the ensuing 24 hours. In the case of the mare-serum hormone (Cutter's gonadin), 10 Cole units were administered per rat in a single injection. The sheep pituitary gonadotropin and the human pregnancy prolan, which were the stable powders previously used in this laboratory (10, 11), were administered in isotonic saline solution, 1 dose per day on 4 consecutive days. The animals were autopsied 96 hours after the initial dose. In addition to ascertaining organ weights and body weight at autopsy, blood pressure readings by the method of Williams, Harrison and Grollman (12), blood non-protein nitrogen, and dry weight of the hearts, were determined in certain instances. In each experiment a comparison was made between litter mates, there being 6 or 7 pairs used for compilation in each experiment. A certain number of the operated rats succumbed during the period of dosage. The corresponding litter mates were then removed from the experiment. Rats which weighed less than 35 grams at the end of the experiment, with their corresponding litter mates, were eliminated from the experiment.

The choice of the level of hormone administered was made by selecting that level which would produce ovarian hypertrophy in the range of greatest sensitivity to dose increment, when administered under optimum conditions as far as they were known. Because of the nature of the results obtained with prolan, the experiment was repeated with double and eight time the dosage level. The conditions chosen for administration of the hormones were those used in earlier experiments (3, 4) so that a basis of comparison with these experiments is afforded.

Condition of nephrectomized rat. It will be noted that while the body weight of the nephrectomized rat is 81 to 84 per cent that of the intact rat, the heart weights are not proportionally decreased. Determination of the dry weight of 6 control hearts and 6 hearts of operated rats gave values of 21.5±0.15 and

21.9±0.19 mgm., respectively. The increase in heart weight:body weight ratio in the nephrectomized rats is indicative of a true cardiac hypertrophy. The increase in blood pressure, which is doubtfully significant, is in line with the observations of Chanutin and Ferris. The consistent rise in the non-protein nitrogen of the blood establishes the condition of renal impairment. Kidney hypertrophy even in the 4-day period is noted, though this may be due in part to hydration.

Ovarian and adrenal weight changes. Since the body weight of the nephrectomized rats was 81 to 84 per cent of that of the intact rats at the time of autopsy, the nephrectomized rats received relatively more hormone on a weight

TABLE I

Influence of nephrectomy upon the response to the gonadotropin in immature female rats

BLOOD PRESSURE	BLOOD NPN MEAN*	TREATMENT	GONADOTROPIN AND DOSE TOTAL PER RAT	BODY WT. MEAN	OVARIAN WT. MEAN	KIDNEY WT. MEAN	HEART WT. MEAN*	ADRENAL WT. MEAN	UTERINE WT. MEAN
mm. Hg	*mgm %*			*gm.*	*mgm.*	*gm.*	*mgm.*	*mgm.*	*mgm.*
	47 (4)	Control intact		51 ±1.0	16 ±1.0	0.79 ±.02	245 (4)	18 ±2.6	16 ±0.9
	46 (4)	Intact	Pituitary 1 mgm.	49 ±1.6	17 ±0.9	0.79 ±.02	230 (4)	19 ±1.1	25 ±4.5
	71 (4)	Nephrectomized		43 ±1.8	14 ±1.2	0.37 ±.01	240 (4)	17 ±0.7	16 ±1.2
	79 (4)	Nephrectomized	Pituitary 1 mgm.	41 ±1.2	25 ±3.1	0.39 ±.02	243 (4)	19 ±0.9	37 ±4.1
87 ±3	34 (5)	Intact	Mare serum 10 u.	47 ±2.0	53 ±4.4	0.72 ±.03	258 ± 8	16 ±0.6	
99 ±4	60 (5)	Nephrectomized	Mare serum 10 u.	38 ±2.4	73 ±8.3	0.30 ±.01	269 ±19	25 ±2.2	
	44 (5)	Intact	Prolan 0.3 mgm.	52 ±2.2	32 ±2.7	0.82 ±.07	258 ±10	17 ±0.4	
	76 (5)	Nephrectomized	Prolan 0.3 mgm.	43 ±2.1	60 ±5.4	0.34 ±.03	255 ± 9	17 ±1.0	
		Intact	Prolan 0.6 mgm.	53 ±2.7	41 ±3.4	0.81 ±.03	273 ±17		
		Nephrectomized	Prolan 0.6 mgm.	41 ±2.5	76 ±8.7	0.29 ±.02	230 ±13		
		Intact	Prolan 2.4 mgm.	50 ±1.2	46 ±2.2	0.72 ±.02	247 ±10	18 ±0.5	
		Nephrectomized	Prolan 2.4 mgm.	40 ±0.7	76 ±9.3	0.23 ±.01	235 ± 9	23 ±1.6	

* The figures in parentheses indicate the number of animals used to obtain the particular data recorded. When not stated the number is 6 or 7. The statistic is the standard deviation of the mean.

basis. A 20 per cent increase in mare serum administration to intact rats over the 10 units administered would produce a 20 mgm. increase in ovarian weight (13). The result obtained in the nephrectomized rats which received mare serum hormone could, therefore, be accounted for by a higher level of hormone on body weight basis. Under the conditions of dosage in the prolan experiment, a 20 per cent increase of prolan dosage to the intact rats would only produce a 2 to 3 mgm. increase in ovarian weight (1). The 28 mgm. increase on the low dose and the 35 mgm. increase on doubling the dose obtained in the operated rats is, therefore, marked and highly significant. However, on increasing the dose still further (eightfold the smaller dose), no further augmentation was noted above the 35 mgm. increase. Even a schedule of divided dosage employing 5 instead of 1 dose per day increases the ovarian response to prolan in intact rats

by only 12 mgm. (2). Administration of the insoluble copper compound produced an increase of 15 to 20 mgm. The pituitary hormone given in these experiments in soluble form once per day and at a 1 mgm. level (total) produced no significant increase in ovarian weight in intact rats and an 11 mgm. increase in nephrectomized rats. Five divided doses per day of the same total dose of hormone, however, produce 50 mgm. ovaries in intact rats (14), administration as the insoluble copper combination produces 100 mgm. ovaries in intact rats, and administration in soluble form to thyroidectomized (4) animals produces 28 mgm. ovaries. It requires 4 mgm. total dose administered once per day for 4 days to produce 28 mgm. ovaries (14). Nephrectomy and thyroidectomy effect the response to the pituitary hormone to approximately the same degree.

There is no indication that nephrectomy influenced the action of endogenous hormone.[2] The data in the table for ovarian and uterine weights for the non-treated nephrectomized rats are within the range of the control data. It is, however, true that the amount of pituitary hormone which will produce specific augmentation of prolan is barely detectable in intact rats under the conditions of our experiment (2). The largest ovarian weight produced by prolan in any of the intact rats was 62 mgm. In 3 nephrectomized rats, ovarian weights over 110 mgm. were produced through administration of prolan. It will be noted that the absolute increase in ovarian weight in the nephrectomized rats, which received prolan, above that produced in the treated intact rats averaged approximately 30 mgm. at each dose-level covering an eightfold dosage range.

Adrenal weights were not significantly changed by nephrectomy or by administration of any of the gonadotropins to intact animals; they were, however, significantly raised in nephrectomized rats by administration of mare-serum hormone and by the large dose of prolan, but not by the small dose of prolan nor by the pituitary hormone. The significance of this finding is not understood.

SUMMARY

Twenty-two-day-old rats were partially nephrectomized by the technic of Chanutin and Ferris. Four days later body weight was approximately 20 per cent below that of litter-mate controls, heart weight was equal to that of controls; the blood non-protein nitrogen was elevated 30 mgm. per 100 cc., and the blood pressure was slightly elevated. Solutions of sheep pituitary gonadotropin, of prolan, and of purified pregnant mare-serum hormone were administered to operated and to litter-mate intact rats.

[2] It is well known that whereas the mare serum and pituitary gonadotropins produce ovarian hypertrophy in the hypophysectomized rat, prolan has a greatly diminished effect. It has been assumed that the action of prolan in the intact rat is due to a synergism produced by the presence of endogenous hormone. In the present experiments an attempt was made to study the action of prolan in the hypophysectomized partially nephrectomized rat. It was found that when the two operative procedures were performed within 48 hours the mortality was high or the surviving animal lost so in body weight that results would have little significance. The results, nevertheless, suggest the possibility that a lowered renal threshold in the hypophysectomized rat may be a factor in the lack of ovarian response.

In the partially nephrectomized rats, the augmented response to pituitary hormone was of the same order as that produced by thyroidectomy, and much less than that produced in intact rats by divided dosage or administration of an insoluble compound. The augmented response to mare-serum hormone could be accounted for by body-weight difference (similar to the influence of thyroidectomy). The augmented response to prolan, which was 175 to 240 per cent, was greater than that produced by divided dosage, thyroidectomy, or administration of an insoluble form. Prolan was able to produce ovaries weighing more than 100 mgm. at the age of 26 days in partially nephrectomized rats.

The well known failure of prolan, in contrast to sheep pituitary and mare-serum hormones, to produce increases in ovarian weight of intact rats proportional to increases in level of hormone administered is interpreted as due to a low renal threshold for prolan.

Less than 90 per cent of injected sheep gonadotropin was recovered in the urine of the mature rat during a six-hour period.

Hypertrophy of the adrenals in nephrectomized but not in intact rats was produced following gonadotropin administration under certain conditions.

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HISTOLOGICAL EFFECTS IN RATS RESULTING FROM ADDING RUBIDIUM OR CESIUM TO A DIET DEFICIENT IN POTASSIUM

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There is a good deal of evidence that Rb and to a lesser extent Cs can replace K in certain physiological processes. Ringer (1) noted that Rb corresponded to K in its action on the frog's heart, while Cs differed in that it resembled more closely the effect of Ba. Clark (2) showed that Rb may be substituted in place of K for the physiological activity of certain isolated organs; Cs was found to be an imperfect substitute. Loeb (3) reported that both Rb and Cs would replace K in solutions required for the development of sea urchin eggs into swimming blastulae. Using rats placed on a synthetic K low diet to which Rb or Cs had been added, Mitchell et al. (4) found that after several weeks the animals became hyperirritable and died shortly afterwards in violent tetanic spasms. Similar changes were noted even when K was added to the diets containing Rb or Cs. Chemical analyses revealed significant amounts of Rb and Cs in the heart and skeletal musculature, although no control studies were made. Heppel and Schmidt (5) have partially confirmed these observations of Mitchell et al. (4) and have concluded that "for a certain time in the growth period of the rat there is some physiological replacement of K by Rb." *In vitro* experiments by Mann et al. (6) have shown that Rb and to a less extent Cs acted as K in bringing about an increased formation of acetylcholine by respiring brain tissue.

Physiological (7) as well as anatomical changes (8, 9) have been observed in animals on diets extremely low in K. Necroses of both myocardial fibres and renal tubular epithelial cells have been noted in several species. The present experiments were designed to find out whether or not the anatomical integrity of the myocardium and kidney could be maintained when Rb or Cs were substituted for K in a diet extremely deficient in the latter element.

Experimental. The basal K-deficient diet used in this study was reported by Orent-Keiles and McCollum (10). It consists of lactalbumin, 10.0; wheat gluten, 4.0; gelatin, 4.0; sweet butter fat, 8.0; K-free salt mixture, 4.6; choline hydrochloride, 0.02, and sucrose (Dyno) to make 100 grams. Fifteen drops of cod liver oil are added to each kilogram of diet. Instead of using liver concentrate as the source of the B group, crystalline vitamins[1] were administered orally twice a week. Each animal received the following amounts per week: thiamine chloride, 0.2 mgm.; riboflavin, 0.28 mgm.; calcium pantothenate, 1.0 mgm.; pyridoxine, 0.2 mgm., and nicotinic acid, 1.0 mgm. Alpha tocopherol, 1.0 mgm. per animal, was given weekly. The substitution of the crystalline vitamins for

[1] Furnished through the courtesy of Dr. D. F. Robertson, Merck and Company.

liver concentrate produced a much more acute deficiency than we have observed heretofore.

Albino rats, weighing 35 to 50 grams, were used. The following are the modifications of the diet made by adding the appropriate salts to the basal ration, together with the number of animals placed on each diet: low K-group, basal diet alone, 6 animals; control group, basal diet plus 0.85 per cent KCl, 5 animals; Rb-substituted group, basal diet plus 0.5 per cent RbCl, 9 animals; Rb-control group, basal diet plus 0.5 per cent RbCl and 0.85 per cent KCl, 3 animals; Cs-substituted group, basal diet plus 0.5 per cent CsCl, 9 animals; Cs-control group, basal diet plus 0.5 per cent CsCl and 0.85 per cent KCl, 3 animals. The basal ration contains about 0.001 per cent K and the control diet contains about 0.44 per cent K. The following percentages of Rb or Cs were used respectively, 0.35 per cent and 0.39 per cent.

The animals were placed in screen bottom cages. Food and distilled water were given *ad libitum.* Microscopic sections of the heart, striated muscle and kidneys were made in all the animals. In addition, sections of all the tissues were made in several representative animals from each group.

RESULTS. *K-deficient group.* The animals on the basal diet (0.001 per cent K) grew poorly and died within 4 weeks (average 25 days). All showed necrotic foci in the myocardium and changes in the renal tubular epithelium similar to those we (9) have described elsewhere. The other tissues appeared to be normal.

Control group. The animals whose diet was supplemented with K (0.44 per cent) grew well. No changes were found in the heart or kidneys.

Rb-substituted group. In these animals growth was far better than in the K-deficient group but poorer than the controls. After being on the diet for about 10 days they became hyper-irritable. When touched they jumped away and cried. At this time it was found that auditory stimuli (rattling of keys, compressed air blast) produced a characteristic and uniform response. The animal first began to tremble, then ran wildly about the cage for 5 to 10 seconds. It then fell on its side with fore and hind legs extended and paws clenched. Though the extremities were spastic, there were fine muscular tremors. In a minute or so these became coarser and gave way to a disappearance of the spasticity while the rat lay seemingly exhausted. The entire episode lasted 3 or 4 minutes. The animals did not die during the first attack but survived a number, one as many as 7 on successive days. The average survival time was 16 days. Those that were observed to succumb did so following an attack.

On microscopic examination no lesions were found in the myocardium of any of the animals. No changes were found in the kidneys of this group of rats, nor were there lesions in the other tissues including the brain.

Rb-control group. These animals failed to grow as well as the Rb-substituted group; their growth was better than the animals on the basal diet. The average survival time was 14 days. No fits such as were observed in the Rb-substituted group were observed in these animals.

Microscopically there were lesions in the myocardium in all 3 of the animals of this group. In 2 the changes were extensive while in the remaining rat the

changes were less severe. In the kidneys and other tissues no changes were observed.

Cs-substituted group. In these animals growth was poorer than in the Rb-supplemented rats. Two of the 9 exhibited fits similar to those of the Rb group. These 2 were among the longest lived animals. The average duration of life was 12 days.

Microscopically there were lesions in the myocardium of 6 of the 9 animals. The kidneys of 3 animals also showed changes similar to those encountered in K deficiency. Those animals dying earliest failed to show myocardial or renal damage.

Cs-control group. These animals grew very well for the first 3 weeks, a little better even than the controls. At this time their growth became stationary and in the last week they lost weight. Coincident with this growth disturbance the rats became hyper-irritable and during the last few days of life exhibited the

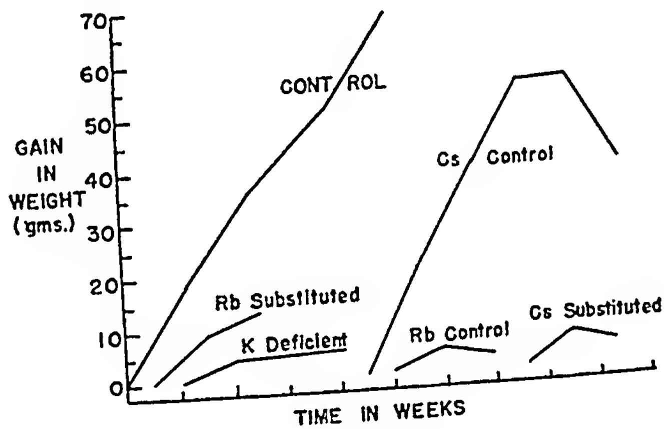

Fig. 1. Growth curves of various groups of animals

same fits that we had observed in the other animals. One striking thing about them was an extraordinary rigor present at death.

Microscopically no lesions were found in the heart and kidneys of these animals. The other tissues likewise showed nothing.

Discussion. When Rb was added to a K deficient diet, myocardial and renal lesions usually associated with K deficiency failed to appear. When Cs was added to the diet, renal changes were encountered in 3 of 9 and myocardial necroses were present in 6 of the 9 animals. Thus it would seem that during the period of observation, at least, Rb was able to replace K and so prevent the renal and myocardial changes, while Cs acted similarly but to a much less extent. The presence of lesions in the Rb control group raises the question as to whether this element is acting as a poison or whether it may be replacing K in the cell. Chemical determinations only can answer this question. In the case of Cs, lesions were not found in the animals dying earliest. It might be argued that there must be a certain time period before changes can take place and that their appearance might be modified by the rate of growth of the animals. On

this diet, however, we have evidence (13) that indicates that even when the food intake of the animals on the low K-diet is restricted so that they gain no weight, lesions appear after 11 days on the diet. Although we have no chemical proof that these elements had passed into the myocardial fibers or renal tubular epithelial cells, studies of Mitchell et al. (4) in the case of the heart, make this seem likely. It is interesting to point out that the main route of excretion of both these elements is via the kidneys (11, 12) and this may help explain the protective effect on this organ.

The seizures which were observed in all the animals on the Rb-substituted group, in a few of the Cs-substituted group, and in all the rats of the Cs-control group, are similar to those described by Mitchell et al. (4) and Heppel and Schmidt (5). Their cause is not clear; no histological changes in the nervous system were found. The fits seem to be identical to those described as specific in Mg deficiency (14), and following the deprivation of pyridoxine in rats (15).

From the effects on growth it seems that, in the proportions used in the diets, when K was present Rb acted as a poison while the reverse was true when K was absent. This observation is similar to what Mitchell et al. (4) found but contrary to that reported by Heppel and Schmidt (5), who observed good growth when K was added to the Rb-containing diet. It is not unlikely that the discrepancy is due to the different proportions of K and Rb in the respective diets. Our dict contained 0.44 per cent K and 0.35 per cent Rb. That of Mitchell et al. (4) was computed as containing 0.9 per cent Rb, the K content was not stated while the Heppel and Schmidt (5) diet contained 0.56 per cent K and 0.28 per cent Rb. The growth of the Cs-substituted group was poor while during the corresponding time the Cs-control animals exhibited vigorous growth for a time; with the onset of fits the growth curve began to fall.

The rôle, if any, which Rb and Cs may play in normal physiological processes is not at all clear; these elements are not considered to be indispensable ones. It should be pointed out, however, that Sheldon and Ramage (16) found Rb to be widely distributed in human tissues and to occur in highest concentration in the heart and skeletal musculature. Scott and Canaga (17) have found, of all tissues examined, Cs is present in the retina of oxen. The significance of this is obscure.

SUMMARY

Microscopic studies were made of the hearts and kidneys of rats that had been placed on a low K-diet to which either Rb or Cs had been added. Whereas myocardial and renal necroses appeared in control animals in the low K-diet, the addition of Rb prevented their appearance. Cs partially protected the kidney and to even a lesser extent the heart. Peculiar fits such as have been described by others were observed when Rb and Cs were added to the diet.

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THE INFLUENCE OF ATROPINE ON THE ATROPHY OF DENERVATED SKELETAL MUSCLE OF THE MONKEY (MACACUS RHESUS)

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On the basis of a study of the dynamics of the chemical changes in denervated muscles of rats (1) we have previously postulated that the unceasing fibrillary activity of the muscle fibers may account for the atrophy which follows nerve section. There is reason to believe that the fibrillation is due to a sensitization of the denervated muscle to the minute amounts of acetylcholine normally present in the body fluids (2). We therefore administered atropine to rats on which nerve section had been performed, and showed that, under our experimental conditions, this drug was able to reduce the degree of atrophy by 39 per cent as compared to untreated control animals (3). The dose of atropine which was effective in our experiments with rats was high as judged by ordinary pharmacologic standards, but most of the animals maintained their body weights. However, the rat is notoriously resistant to atropine and there is little information available as to species difference in susceptibility to this drug. In the present work we tested the effect of atropine on the atrophy of denervation in monkeys, as a further step towards the possible therapeutic application of the drug in humans.

METHODS. Female monkeys (Macacus rhesus) ranging in weight from 3.0 to 4.3 kgm. were used throughout. One hind limb was denervated by section of both the sciatic and femoral nerves. The other hind limb served as the weight control. Twenty-one days after denervation[2] the gastrocnemii of both hind limbs were removed and weighed immediately. The difference in their weights represented the percentage atrophy suffered by the denervated muscle. In the treated monkeys atropine (sulphate) was administered subcutaneously, twice daily, for the 21-day period. The total dosage per animal per day was varied for different groups of animals, as detailed in table 1.

RESULTS. Table 1 summarizes our data. The average per cent atrophy in the untreated group was 32.5 per cent. Except for the smallest daily dose of atropine, one or more animals in all the treated groups responded with a significant diminution of the degree of atrophy. Regardless of the dose, all animals which did respond to atropine showed about the same inhibition of atrophy, ranging from 26 to 39 per cent. Those monkeys which did not give this response, gave practically no response at all. That is, an "all or none effect" is suggested. However, our data also suggest that with the highest doses used, more of the

[1] Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

[2] In our work with rats we used a 14 day period. Preliminary tests on monkeys showed only a 13 per cent atrophy of control muscles in 14 days.

TABLE 1

Effect of varying doses of atropine upon the atrophy of denervated gastrocnemii in monkeys, 21 days after nerve section

NO. OF MONKEYS	ATROPINE	BODY WEIGHT		PER CENT ATROPHY OF GASTROCNEMIUS	PER CENT DEVIATION† FROM CONTROL	
		Initial	Final		Not significant	Significant
	*mgm./day**	*kgm.*	*kgm.*			
4	0 (Control)	3.5	4.0	33‡		
		3.0	3.6	29‡		
		3.8	4.0	33‡		
		4.0	4.2	35‡		
2	0.5			32	−2	
				28	−13	
2	1.0	3.6	4.0	33	+2	
		3.8	4.2	20		−39
2	2.0	4.0	4.0	35	+8	
		3.0	3.4	22		−32
3	3.0	4.1	4.0	35	+8	
		3.0	3.2	24		−26
		3.5	3.0	22		−32
3	4.0	3.8	4.0	32	−2	
		3.6	4.0	28	−13	
		3.7	3.9	23		−29
3	5.0	3.4	3.8	32	−2	
		4.0	3.6	32	−2	
		4.1	4.0	21		−38
8	10.0	3.8	4.0	35	+8	
		4.3	4.2	31	−5	
		3.0	3.3	31	−5	
		3.8	3.6	24		−26
		3.5	3.9	23		−29
		4.0	4.2	22		−32
		3.5	3.3	20		−39
		3.7	4.0	20		−39
3	15.0	4.1	4.0	24		−26
		3.6	3.8	24		−26
		3.8	3.9	20		−39

* The indicated daily dosage was given subcutaneously in 2 equal portions, and administered from the day after the nerves were sectioned until the day upon which the muscles were removed for examination.

† Plus sign indicates increased atrophy; minus sign indicates decreased atrophy.

‡ Average per cent control atrophy 32.5.

animals exhibited significant inhibition of atrophy. As regards the possible general effects of the atropine, only one animal out of the 30 lost a significant amount of weight during the treatment, and that animal was in the lower dosage range. With this exception all the monkeys exhibited only minor fluctuations in body weight, even at the highest dose levels.

Discussion. The present work confirms in the monkey our previous results and conclusions as to the effect of atropine on the atrophy of denervation in rat muscle (3). This effect is, therefore, not restricted to one species. Moreover, in the monkey, the effective doses of atropine are much smaller on the basis of body weight than in the rat. In rats we used 15 mgm. of atropine per 100 grams body weight, per day (given subcutaneously in two equal parts), while in the present work the highest dose averaged 0.5 mgm. per 100 grams body weight per day. With this dose, and to a lesser extent with the next highest dose, the monkeys were not as bright and active as usual, although no other manifestation of toxicity was evident. The fact that our treated animals maintained their body weights precludes the possibility that the inhibition of atrophy might have been due to a differential loss of weight by the normal and denervated muscles, because of undernutrition.

It is difficult to predict the doses of atropine which might prove effective in human beings. But, considering their bulk as compared to rats and monkeys, it seems quite possible that amounts of atropine which could be tolerated would be effective in inhibiting the muscular atrophy resulting from peripheral nerve injury or anterior poliomyelitis.

CONCLUSION

The muscular atrophy following nerve section in monkeys (Macacus rhesus) can be significantly inhibited by treatment with atropine. This confirms similar results previously obtained on rats, and favors the possibility of a therapeutic application of the drug in humans.

We wish to thank Dr. Philip Lewin for his interest in and his encouragement of this work.

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INFLUENCE OF GELATIN INGESTION ON THE CONCENTRATION IN THE RAT GASTROCNEMIUS OF PHOSPHOCREATINE AND RELATED COMPOUNDS

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In a study of the ageing process close attention was paid to the degree of variability, independent of age, that may be expected in the functions studied. It is only after this issue is elucidated that one can certainly ascribe changes observed to the ageing process. For example, what deviations from average values are to be expected when considerable variations are introduced into the dietary regime?

Fortunately for our purpose, a controversy has been going on as to the effects of amino acetic acid or of gelatin, a protein rich in this amino acid, on the concentration of muscle creatine (1). This offered the dual opportunity of testing on the one hand the stability of the chemical system of muscle tissue when a large amount of a particular amino acid is ingested and on the other hand of testing the assumption that amino acetic acid has some unique rôle in creatinogenesis.

Methods. Adult male rats weighing approximately 200 grams were matched as to size and divided into two groups. Both were fed a stock diet of Purina dog chow, supplemented with green vegetables and powdered skim milk.

To study the excretion of creatine and creatinine, the rats were placed in metabolism cages, two animals in each, for a period of one week before any collections were made. Following this preliminary week, three consecutive 24-hour urine samples were obtained as controls. To the individuals of one group of rats, ½ gram of gelatin in 3 ml. of water was given daily by stomach tube, while to the control group a calorie equivalent of glucose was given in like manner. These supplements were added to the diet for eight days and 24-hour urine samples were collected each day. The urine, free from food and feces, was collected under oil with thymol as a preservative. At the end of each 24-hour period, the cage was washed down with distilled water and the washings added to the urine sample. Aliquots were analyzed for creatine plus creatinine by autoclaving the sample with hydrochloric acid, followed, after neutralization, by the addition of alkaline picrate. Preformed creatine was determined by adding alkaline picrate to fresh urine samples. All color comparisons were made in the Evelyn photoelectric colorimeter.

On the eighth day the animals, having been deprived of food for 12 hours, were anesthetized with nembutal. A gastrocnemius muscle was dissected free and frozen in situ in carbon dioxide snow. The method of sampling the tissue and the procedures employed in the analysis of the tissues have been described (4). Blood was obtained by cardiac puncture.

RESULTS AND DISCUSSION. Many workers have thought that the occurrence of an increased urinary excretion of either creatine or creatinine or both following the administration of various amino acids, other foodstuffs, or hormones, is sufficient evidence of an increased formation of muscle creatine. Beard et al. (2) have reported a marked increase in creatine and creatinine excretion following the injection of a single dose of glycine. As can be seen from table 1, urinary excretion of these compounds by rats was but slightly altered by a high gelatin diet. Averages of all values for the urines collected revealed an increase of only 0.2 mgm. of creatine per day accompanied by a concomitant decreased excretion of creatinine. Variations of this magnitude may have arisen as the result of the inadequate method of collection. Creatinine is readily converted into creatine in some urine samples on standing, even for short periods of time, at ordinary room temperature (3).

TABLE 1

The urinary excretion of creatine, creatinine and nitrogen per rat before and during gelatin feeding

	Rats 3 and 4		Rats 5 and 6		Rats 7 and 8		Rats 9 and 10		Rats 11 and 12		Rats 1 and 2 not given gelatin
	Before	During	Before	During	Before	During	Before	During	Before	During	
Creatine* mgm. per 24 hours	2.3	4.1	3.7	3.8	4.7	4.1	4.0	4.1	4.4	4.3	3.4
Creatinine mgm. per 24 hours	4.2	4.8	4.5	4.7	8.4	7.4	5.2	4.3	5.7	5.0	3.8
Nitrogen gram per 24 hours	0.17	0.18	0.13	0.17	0.23	0.26	0.25	0.24	0.25	0.25	0.18

* Expressed in terms of creatinine.

Values for the various determinations made on the resting gastrocnemius muscles of 22 rats, fasted for 12 hours, are shown in table 2. Ten of the animals had received $\frac{1}{2}$ gram of gelatin daily for eight days. Of the 22 rats, 6 were from a single litter. Two of these litter mates were in the control group and the remainder in the group receiving gelatin.

Since many of the compounds studied are markedly influenced by even slight activity of the animal, it should be pointed out that these animals were in the resting condition. The concentration of lactate in blood and muscle is regarded as evidence that the animals were in a resting state at the time of sampling. Newman (7) reported respective values of 13 and 22 mgm. per cent lactate in blood and muscle. Average values of blood and muscle lactate in the present study were 10 and 16 mgm. per cent in the gelatin group and 13 and 19 in the control group.

There was no significant difference between the control and gelatin groups in any of the substances studied. In general, agreement was excellent not only

between litter mates, but also among all other rats. A critical evaluation of the influence of the ageing process on the distribution of the phosphate and related compounds has been presented (4).

The two compounds in muscle which are supposed to be affected by the addition of gelatin to the diet are the total creatine and that part of it bound in the form of phosphocreatine. According to Ray et al. (8) gelatin added to the diet had a marked influence on the physical capacity of man. The allaying of muscular fatigue and the increased work output was attributed to the creatinogenic effect of the amino acetic acid portion of the gelatin with the implication that there was an actual increase in phosphocreatine. If phosphocreatine in the muscle increases, it might serve as an additional source of muscular energy.

TABLE 2

Mean composition of adult rat tissue in relation to gelatin feeding*

Total solids and total nitrogen are in terms of grams per cent in wet tissue; all other concentrations are in milligrams per cent.

	RATS ON STOCK DIET	RATS ON STOCK DIET PLUS 3 GRAMS GELATIN DAILY FOR 8 DAYS		RATS ON STOCK DIET	RATS ON STOCK DIET PLUS 3 GRAMS GELATIN DAILY FOR 8 DAYS
Number of animals	12	10	Adenosine triphosphate	38	39
Total solids	25.0	25.5	Hexose phosphates	19	22
Tissue nitrogen	2.1	2.4	Barium insoluble phosphate	77	77
Total creatine	406	396	Glycogen	590	525
Total phosphate	228	232	Muscle lactate	19	16
Acid soluble phosphates	154	155	Blood lactate	17	10
Inorganic phosphate	19	20	Blood sugar	129	132
Phosphocreatine	58	56			

* Eight to nine months old.

Possibly it would facilitate a more rapid resynthesis of adenosine triphosphate, which is believed to furnish the immediate energy for muscular contraction.

No changes in either phosphocreatine or total creatine were found as the result of gelatin ingestion by the rats in this study. The phosphocreatine concentrations were 56 and 58 mgm. per cent respectively for the gelatin and non-gelatin groups, while the total creatine concentrations were 396 and 406 mgm. per cent. This finding that gelatin has no positive effects is in accord with the observations of Horvath et al. (5) and of Robinson and Harmon (9) that gelatin added to the diet of men did not alter their strength or endurance. Knowlton (6) found that gelatin did not affect the strength of the leg muscles of rats.

SUMMARY

The feeding of gelatin, a rich source of amino acetic acid, to adult rats as a supplement to an adequate diet did not influence the concentration in the gastrocnemius muscle of the chemical compounds known to be associated with

muscular contraction. Gelatin exerted no creatinogenic effect. No changes were found in either phosphocreatine or total creatine. There was no evidence on the basis of increased excretion of creatine or creatinine of an increased destruction of muscle creatine.

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THE RELATION OF FOOD CONSUMPTION, HYPOPHYSIS AND ADRENAL CORTEX TO SERUM ALBUMIN METABOLISM IN THE RAT[1]

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In a previous communication (1) it was suggested that the decrease in serum-albumin concentration following hypophysectomy in rats is due mainly to decreased adreno-cortical function. The self-imposed inanition which follows removal of the hypophysis also has an influence. However, that this is of relatively minor importance in the short experimental periods employed was demonstrated (1) by the finding that in intact female rats, subjected to an inanition somewhat more severe than that suffered by the operated animals, the serum-protein changes were of much smaller magnitude than those found after hypophysectomy. Moreover, hypophysectomized rats treated with adrenocortical substances were found to maintain their serum albumin at a fairly normal level even though the loss of body weight was as great as in untreated, operated animals.

These findings indicate that under the influence of adrenocortical hormone the rat is able to maintain its serum-albumin level, if necessary, at the expense of other body proteins. However, if adrenocortical function is decreased or entirely absent, the maintenance of the normal serum-albumin level by utilization of endogenous protein does not appear possible. It was, therefore, of interest to know whether animals deprived of most or all of their adrenocortical function are able to maintain the serum-albumin level if sufficient food is supplied by mouth, or in other words, whether adrenocortical intervention is necessary for the formation of serum albumin from exogenous protein. To this end we have determined the serum-protein levels of normal and of hypophysectomized rats supplied with adequate amounts of protein by stomach tube. The results were compared with those obtained from similar animals eating *ad libitum*. In addition, to check our previous findings in female rats, we determined the serum-protein levels in intact male rats subjected to severe inanition.

The results of these investigations, reported in this communication, show that the serum albumin of the hypophysectomized rat remains at very nearly the normal concentration if the food intake is maintained at a sufficiently high level.

METHODS. Adult male rats were selected and divided so that the different experimental groups matched approximately in age and weight. Two of the groups were hypophysectomized while the other two remained unoperated. One of the hypophysectomized and one of the intact groups received no treatment of

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any sort, being allowed to eat the regular stock diet[2] *ad libitum*. The other two groups, one of operated and one of intact animals, were fed by stomach tube, usually beginning about 36 hours after the operation. The tube feeding was done twice daily by a method described elsewhere (2), using increasing amounts of the food mixture [3] during the first few days so as to minimize danger of food shock. These animals therefore lost weight for several days until the amount of food given was sufficient to supply the caloric requirements. The same amount of food was administered to the normal and operated animals regardless of weight or other factors.

In addition to the above, another group of intact male rats was subjected to a reduced food intake (using the regular stock diet) so as to simulate the weight loss suffered by the hypophysectomized animals eating *ad libitum*.

All the experiments were conducted over a period of 3 weeks. At the end of this period the animals were bled from the heart and the sera analyzed by methods previously described (1). The completeness of hypophysectomy was verified at autopsy by careful examination of the pituitary region with the aid of a high power dissecting microscope. In addition, the testes, seminal vesicles, adrenals

[2] The stock diet used for our rat colony is composed of the following ingredients which are thoroughly mixed and fed *ad libitum* as the dry mixture. A supplement of fresh lettuce is fed once weekly and water is available to the animals at all times.

Ground yellow corn	15 parts (by weight)
Ground whole wheat	15 parts
Ground hull-less oats	15 parts
Ground whole barley	15 parts
Soy bean meal	15 parts
Meat scrap	10 parts
Whole milk powder	5 parts
Skim milk powder	5 parts
Ground alfalfa meal	2 parts
NaCl	2 parts
$CaCO_3$	0.5 parts

[3] The liquid diet used for tube feeding in these experiments is prepared as follows:

Whole milk powder	300 gm.
Dried egg albumin	40 gm.
Dried irradiated yeast	50 gm.
Cellu flour	60 gm.
Salt mixture (Osborne Mendel)	10 gm.
Glucose	115 gm.
Mazola oil	45 cc.
Wheat germ oil	5 cc.
Cod liver oil	5 cc.
Vitamin B syrup[4] (Abbott)	15 cc.
Water	500 cc.

The glucose is dissolved in the water and the solution is added to the mixed dry ingredients. The mixture is stirred with an egg beater, adding the oils and the vitamin B preparation. Stirring is continued until the mixture is homogeneous. This mixture was administered in daily doses of 24 cc., divided into two feedings. This amount (24 cc.) is calculated to contain 2.9 grams protein, 5.5 grams carbohydrate and 3.2 grams fat as available foodstuffs. The yeast was omitted from the calculations.

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and thyroids were dissected and weighed. The atrophy of these organs during the three-week post-hypophysectomy period is sufficiently great to allow dependable judgement as to the completeness of pituitary removal.

RESULTS AND DISCUSSION. The changes in body weight of the different experimental groups are charted in figure 1. It is evident that the tube-fed hypophysectomized rats, after the immediate 2 or 3 day postoperative period, gain weight at a rate identical with that of the intact animals given the same amount of food. The greater loss of weight suffered by the operated animals during the first 2 days is probably due to the effects of anesthesia, surgical procedures, etc. It is of considerable interest to note that the rate of weight increase of the

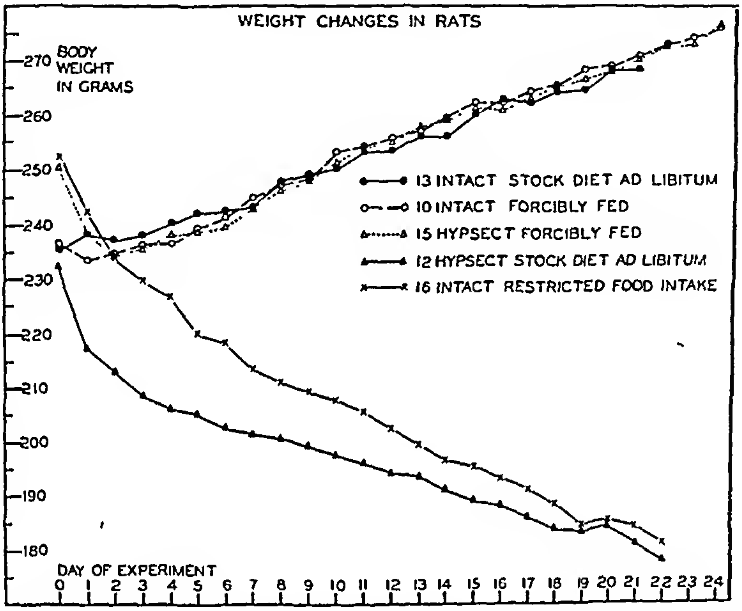

Fig. 1. Relation of food consumption, hypophysis and adrenal cortex to serum albumin metabolism in the rat.

force-fed animals, hypophysectomized as well as intact, is identical with that of intact animals eating the stock diet *ad libitum*. It would be of interest to know whether the composition of this weight increment is the same for the 3 types of animal. That this is so is indicated by the findings of Samuels et al. (3), but would not be expected in the light of the work of Lee and Ayres (4).

The analytical data obtained from the sera of the above described animals are summarized in table 1. The changes found in the hypophysectomized animals eating *ad libitum* (1, C) agree well with those previously reported (1) for such animals, being a marked decrease in serum albumin and an equally marked increase in serum-globulin concentration. Likewise, severe inanition in intact male rats produces changes (1, B) similar to those previously found in starved female rats. These changes are qualitatively similar but quantitatively of much less magnitude than those seen after hypophysectomy. This indicates that the hypophysectomized rat, deriving a considerable proportion of its caloric requirements from endogenous sources (as shown by the marked loss of body

weight), is not able to replenish or replace its serum-albumin stores. On the other hand, the intact rat, if forced to subsist to an equal extent on its endogenous resources as a result of curtailed food intake, is able to maintain its serum-albumin concentration at a more nearly normal level.

This difference in ability to maintain the serum albumin level disappears if an amount of food, approximately isocaloric with that which is voluntarily consumed by the intact rat, is administered to the hypophysectomized as well

TABLE 1

Effect of various treatments on serum protein levels of the rat

TREATMENT	NO. OF RATS	AGE AT BLEEDING	DURING EXPT.		CELL VOLUME	NON-PROTEIN NITROGEN	TOTAL PROTEIN	ALBUMIN	GLOBULIN	ALBUMIN / GLOBULIN
			Time	Wt. chg.						
		days	*days*	*per cent*	*per cent*	*mgm. per cent*	*per cent*	*per cent*	*per cent*	*ratio*†
A. Normal—stock diet ad lib.	18	112–138	15–22	+12.2±1.2	44.6±0.5	33.8±1.0	6.04±0.07	4.01±0.03	2.04±0.06	1.99±0.06
B. Intact—restricted food intake	16	124–134	20–22	−27.0±0.9	45.5±0.7	35.6±1.2	5.63±0.08	3.57±0.07	2.06±0.08	1.78±0.08
	per cent change from normal				+2.2	+5.3	−6.8	−11.0	+1.0	−10.5
	P* (vs. normal)				0.30	0.25	<0.01	<0.01	0.80	0.05
C. Hypsect.—stock diet ad lib.	12	112–129	21–22	−21.1±2.0	40.9±1.4	46.5±1.5	5.57±0.15	3.07±0.07	2.50±0.10	1.24±0.05
	Per cent change from normal				−8.3	+37.6	−7.8	−23.4	+22.6	−37.7
	P* (vs. normal)				<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
D. Intact—force-fed	10	128–138	19–24	+15.4±1.7	42.7±3.5	30.0±1.7	5.85±0.15	3.70±0.08	2.15±0.11	1.76±0.09
E. Hypsect.—force-fed	15	128–138	19–24	+7.9±1.0	36.5±1.0	44.6±0.7	6.20±0.10	3.52±0.08	2.68±0.07	1.32±0.04
	Per cent change from normal force fed				−14.5	+48.7	+6.0	−4.9	+24.7	−25.0
	P* (vs. intact force fed)				<0.01	<0.01	0.07	0.15	<0.01	<0.01
	Per cent change from hypsect. ad lib.				−10.8	−4.1	+11.3	+14.7	+7.2	+6.5
	P* (vs. hypsect. ad lib.)				0.02	0.25	<0.01	<0.01	0.15	0.22

The ± values are for the mean deviation of the mean, calculated as $\sigma_m = \sqrt{\dfrac{\Sigma d^2}{n(n-1)}}$

*P expresses the probability that the difference between two means is due to random sampling (11). A P value of 0.05 or less is frequently taken to indicate statistical significance.

† The values given for the A/G ratio are the means of the individual A/G ratios rather than the ratio of the mean albumin to mean globulin.

as to the normal animal. As shown by the data of table 1, D, intact rats receiving their entire food supply by stomach tube yield results comparable to those obtained from normal animals eating *ad libitum* (table 1, A). Hypophysectomizde rats, given this same amount of food by stomach tube, maintain the serum-albumin concentration at approximately the normal level (table 1, E). The ratio of albumin to globulin, however, is low because the forced feeding does not prevent the sharp increase in serum globulin which is found after hypophysectomy or thyroidectomy in the rat (1) and in the dog (5). It may be noted that the hematocrit level of the forcibly fed hypophysectomized animals is considerably lower than that of the intact rats. Although the reason for this is not known to us, it is suspected that it is due to decreased hemopoiesis (6). If, however, the

low hematocrit is due to a hemodilution in the sense of increased plasma volume, then the serum-albumin maintenance is even better than shown by the figures.

These results are interpreted to mean that little or no adrenocortical hormone is required for the maintenance of the serum-albumin level if sufficient food is supplied to the animal. However, if the animal is forced to subsist wholly or in part on its endogenous resources, adrenocortical or hypophyseal biocatalysis is required for the maintenance of the serum-albumin level. Previous experiments (1, 7) provide evidence that the necessary hormonal action is adrenocortical rather than hypophyseal.

The situation with regard to serum-albumin maintenance, therefore, appears to be analogous to but more extreme than that concerning maintenance of carbohydrate stores. It has been adequately shown (8, 9) that adrenalectomized or hypophysectomized animals are able, under ordinary circumstances, to maintain fairly normal carbohydrate stores despite the sharply decreased food consumption. Only under conditions of stress, when carbohydrate must be rapidly formed from endogenous protein, does the gluconeogenic mechanism break down.

Similarly, serum albumin apparently can be manufactured satisfactorily from exogenous protein with the reservation that the supply of this raw material must be considerably greater than that which the operated animal voluntarily consumes. When the supply of exogenous protein is decreased, as in the hypophysectomized or adrenalectomized animal eating *ad libitum*, the replacement of serum ablumin is greatly impaired. However, if the animal has an adequate supply of adrenocortical hormone it apparently is able to maintain its serum-albumin level at the expense of other body proteins, at least for periods as long as those studied in the present experiments. That the mechanism may ultimately break down, at least in some species, is known (10).

The author gratefully acknowledges the important technical assistance of Miss Shirley Mischler and Miss Betsy Conant.

SUMMARY AND CONCLUSIONS

1. Following hypophysectomy, rats voluntarily decrease their food consumption and show a marked loss of body weight. Concomitantly, there occurs a marked decrease in the serum-albumin concentration.

2. Starvation of intact rats to simulate the body-weight loss of the hypophysectomized animals also produces a decrease in serum albumin but this is of much less magnitude than that occurring in the operated animals.

3. If adequate quantities of food are forced into hypophysectomized adult rats by stomach tube, they can be caused to gain weight at a rate comparable to that resulting from *ad libitum* feeding of normal animals. In such force-fed hypophysectomized rats, the serum-albumin concentration is maintained at the normal level.

4. These and previous findings indicate that with adequate adrenocortical function the rat is able to maintain its serum-albumin concentration, at least for periods as long as three weeks, even when forced to subsist partially on its

own tissues. In the event that adrenocortical function is decreased or absent, the rat is unable to maintain its serum albumin at the normal level unless it is supplied with quantities of food considerably larger than hypophysectomized or adrenalectomized animals voluntarily consume.

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THE ANTAGONISTIC EFFECT OF LIPOCAIC AND THE ANTERIOR PITUITARY ON FAT METABOLISM[1]

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There is now general agreement that the activity of raw pancreas in preventing the deposition of fat in the livers of depancreatized dogs is not due to lecithin, choline, betaine, the enzymes of the pancreatic juice or to the general lipotropic action of protein. This activity is due to a specific substance, lipocaić, and the evidence indicating that this is a hormone distinct from insulin has been enumerated by Dragstedt (1). The suggestion by Best (2) that lipocaic is not a hormone but rather a specific dietary factor is probably a matter of definition.

The mode of action of lipocaic, except that in a general way it is concerned with the transport and utilization of fat, has remained relatively obscure. The characteristic changes in lipocaic deficiency: loss of body fat, accumulation of liver fat in large amounts, and hypolipemia are observed in depancreatized dogs in whom the other specific defects are corrected by the administration of pancreatic juice and insulin. These findings suggest a transport of depot fat to the liver and accumulation there due either to the increased rate of mobilization of fat or to an inability of the liver to further metabolize it. The administration of lipocaic corrects this metabolic abnormality in the depancreatized dog.

Abnormalities in fat metabolism similar to those seen in the insulin-treated depancreatized dog can be produced in smaller animals by the administration of certain anterior pituitary extracts. The first extract of this kind was prepared in 1930 by Burn and Ling (3). They termed the material ketogenic hormone because when they administered it to rats an increase in the urinary excretion of ketones was observed. The action of this substance was further studied by Anselmino and Hoffman (4) who found an accompanying increase in the blood ketones and in 1934 by Steppuhn (5) who showed that there was an increase in the fat content of the liver of rats receiving injections of the ketogenic hormone. Best and Campbell (6) studied the production of this type of fatty liver. In 1936 they confirmed the previous observations and noted that accompanying the increase in liver fat there was a decrease in the amount of body depot fat in fasted animals given the extract and that this decrease was greater than the decrease seen in the control fasted animals. The fat lost from the depots of the body was found to account for about one-half of the lipid recovered on analysis of the liver.

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[2] Submitted by Dr. Julian to the University of Chicago in partial fulfilment of the requirement for the degree of Ph.D. in surgery.

Best (7) had previously confirmed earlier work showing that large doses of choline would prevent the fatty liver of depancreatized dogs. Consequently, he tested the effect of choline on the fatty liver produced by anterior pituitary extracts and found no effect.

The purpose of the present study was to observe the effect of administration of lipocaic on the fatty liver produced by the injection of pituitary extracts. Further experimental work suggested by the results of this study is also reported.

This type of experiment had previously been made by McKay and Barnes in 1938 (8). These workers tested the effect of both choline and lipocaic on the ketogenic hormone fatty liver. The conclusion from their data was that neither substance had any effect. However, the pancreas extract was given in amounts which seemed to us to be too small to expect definite activity. They prepared lipocaic according to the method given in 1936 by Dragstedt, Prohaska and Harms (9), and, using rats, administered an amount of this extract equivalent to 8.7 grams fresh pancreas per square decimeter of body surface daily. This is approximately 30 grams fresh pancreas per day for a rat weighing 175 grams.

In the present study the lipocaic was purified for small animal work by the glacial acetic acid-ether method reported by Clark, Vermeulen, Donovan, and Dragstedt in 1939 (10). This method yields an average of 100 mgm. of extract from 200 grams of raw pancreas. In the work to be presented, daily doses of this extract ranging from 25 to 250 mgm., equivalent to 50 to 500 grams of raw pancreas, were given each animal. The preparation of the ketogenic hormone followed the method of Best and Campbell.

Three groups of young female guinea pigs weighing 300 to 375 grams were used. The initial weights were determined. All animals received only water by mouth during the 72-hour experimental period. Each animal in the three groups received an intraperitoneal injection after 24 hours and again after 48 hours of fasting. They were killed 24 hours after the second injection.

The animals in the control group received injections of 3 cc. of saline. The animals of the second group (group B) were given ketogenic hormone in doses varying from 2 to 10 mgm. per 100 grams of body weight. The animals of group C received similar amounts of ketogenic hormone plus total daily doses of 25 to 250 mgm. of lipocaic injected intraperitoneally at separate sites. At the completion of the 72-hour period the loss in the body weight, the final liver weight, and the percentage of lipid in the liver were determined. The total liver lipids and the milligrams of liver lipids per 100 grams final body weight were calculated. The results of these determinations are presented in table 1.

The average loss of body weight was greatest in both groups of animals receiving ketogenic hormone. This weight loss was not affected by the administration of lipocaic. The livers of the animals receiving ketogenic hormone were heavier than those that received additional lipocaic and than those of the control group.

The average percentage of liver lipids was found to be 11.1 in the control animals, 18 in the ketogenic hormone group, and 8.1 in the animals receiving lipocaic with the ketogenic hormone. The total liver lipids followed the same

pattern being 1138 mgm., 2677 mgm. and 1041 mgm. respectively in the three groups. To relate these figures to the sizes of the animals, the milligrams of liver lipid per 100 grams of final body weight are given. These calculated figures show very strikingly the protective action of lipocaic against the effect of ketogenic hormone.

This demonstration of an antagonistic relationship between lipocaic and the pituitary extract, ketogenic hormone, is important from the standpoint of the mechanism of lipocaic deficiency in the depancreatized dog. Does the insulin treated depancreatized dog develop a fatty liver as a result of the action of the anterior hypophysis, unopposed by lipocaic?

TABLE 1

Antagonistic effect of lipocaic on the fatty infiltration of the liver produced in guinea pigs by fasting and the injection of ketogenic hormone

NO. OF ANIMALS	AVERAGE INITIAL WT.	AVERAGE FINAL WT.	AVERAGE LOSS IN WT.	AVERAGE LIVER WT.	PER CENT LIVER LIPID			MGM. TOTAL LIPID IN LIVER			MGM. LIVER LIPID PER 100 GM. FINAL BODY WT.		
					High	Low	Aver.	High	Low	Aver.	High	Low	Aver.
Control group—3 cc. saline intraperitoneally													
	gm.	*gm.*	*gm.*	*gm.*									
6	349	298	51	9.9	16.5	5.2	11.1	1575	499	1138	496	172	375
Group B—1.5 to 10 mgm. ketogenic hormone per 100 gm. of animal intraperitoneally													
19	341	274	62	14.8	27.9	11.2	18	4525	1710	2677	1932	540	1011
Group C—1.5 to 10 mgm. ketogenic hormone per 100 gm. plus total dose of 25 to 250 mgm. of lipocaic preparation													
21	349	293	60	12.7	12.0	4.3	8.1	1480	425	1041	610	175	364

In order to examine further the rôle of the hypophysis in the fatty liver production in pancreatectomized dogs, a series of animals was first hypophysectomized and then depancreatized.

The results of this study are given in table 2. The dogs, nos. 1 to 6, survived the second operation from eleven to forty-two days. At autopsy the livers showed uniformly a marked increase in fat content, ranging from 22 to 42 per cent.

Dogs 7 to 10 were subjected to liver biopsy seven to forty-nine days after pancreatectomy. After biopsy the dogs were given lipocaic by mouth in doses known to cure fatty liver in pancreatectomized dogs. Following lipocaic administration for eight to one hundred and five days the dogs were sacrificed, usually at an attempted second biopsy, and a uniform decrease in liver fat observed. The liver of one of these dogs (no. 10) was biopsied after thirty-five days following pancreatectomy at which time the liver assay showed 43 per cent lipid. A second biopsy forty-one days later, lipocaic being given in the interval, showed 12 per cent fat in the liver. The dog died thirty-one days

after this second biopsy, and at this time a normal fat content of the liver was found.

The depancreatized, hypophysectomized dog develops a severe fatty liver in as short or shorter time than the dog with pancreatectomy alone. This fact has been infrequently observed in this preparation because most investigators using the Houssay animal have fed raw pancreas, containing lipocaic, as a food supplement.

This rapid appearance of the fatty liver in the Houssay animal indicates that the fat metabolism factor of ketogenic hormone is not the only factor in the

TABLE 2

Development of fatty infiltration of the liver in the hypophysectomized-depancreatized dog (Houssay) and response to lipocaic therapy

DOG	OPERATION DATES		DATE OF DEATH	LIVER FAT PER CENT WET WT.
	Hypophysectomy	Pancreatectomy		
				per cent
1	6/4	9/2	10/2	27
2	10/5	10/26	11/19	35
3	11/20	12/16	12/29	22
4	12/15	12/30	2/14	38
5	12/18	2/5	2/20	32
6	10/15	11/4	11/15	42

DOG	OPERATION DATES		LIVER BIOPSY		TREATMENT	LIVER POST-MORTEM	
	Hypophysectomy	Pancreatectomy	Date	Fat		Date	Fat
				per cent			*per cent*
7	11/26	12/3	1/3	32	Lipocaic 16 days	1/10	11
8	12/30	3/31	4/7	12	Lipocaic 8 days	4/15	9.9
						Perforated ulcer	
9	12/24	2/24	4/13	18	Lipocaic 3½ mo.	6/24	11
10	11/19	12/3	1/8	43	Lipocaic 2½ mo.	3/20	Normal liver
			2/19	12			

appearance of fatty liver in the depancreatized dog, though it may play a part in the lipid metabolism of the intact animal.

Discussion. The antagonistic action of lipocaic and certain extracts of the anterior pituitary suggests that under normal conditions the transport of fat between the body depots and the liver is regulated by opposing hormone influences. It seems probable that the anterior pituitary liberates a substance into the circulation which causes a migration of body fat to the liver. Excessive breakdown of this fat results in hyperketonemia and ketonuria. Lipocaic, on the other hand opposes at least a part of this effect and causes the migration of fat to the body depots. Evidence with respect to the effect of lipocaic on the ketonemia and ketonuria resulting from injection of the ketogenic hormone is

not available. While the accumulation of fat in the liver of the depancreatized dog may be in part due to the unopposed action of the anterior pituitary in the absence of lipocaic, the fact that this accumulation occurs with equal rapidity in the "Houssay" animal indicates that this is not the sole mechanism.

CONCLUSIONS

1. The parenteral administration of lipocaic prevents the accumulation of fat in the liver produced by the injection of ketogenic hormone in fasting guinea pigs.
2. The fatty infiltration in the liver due to fasting is also decreased by the administration of lipocaic.
3. The Houssay animal develops the fatty liver of lipocaic deficiency just as rapidly as the depancreatized dog and is equally responsive to lipocaic therapy.
4. Some implications of the antagonistic action of lipocaic and the anterior pituitary on fat transport are mentioned.

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STUDIES ON INTRAOSSEOUS INJECTIONS OF EPINEPHRINE

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In the last two or three years blood has been successfully transfused in both animals and human beings by injection into the medullar cavity of long bones. Particularly meritorious work along this line has been published by two sets of American clinical investigators. Morrison and Samwick (1) reported successful intramedullar transfusion not only of blood but of human bone marrow cells, while Tocantins and O'Neill (2) injected whole blood, blood plasma, glucose solution and congo red through the bone marrow. Such hematological experimentation prompted an inquiry by the present author into the feasibility and effects of intramedullar injection of different pharmacological agents on various animals. Fifty experiments were made on guinea pigs, forty on rabbits, thirty on cats, five on dogs and twenty on rats. In many instances such experiments yielded striking results, which have been described in a preliminary paper (3) on the absorption of drugs through the bone marrow. In the present communiication the writer purposes to discuss in detail one drug, epinephrine, which has yielded experimental data of unusual interest to both physiologist and pharmacologist.

METHOD. Intramedullar injections are readily made after some practice by introducing stout needles or trocars into the long bones of rabbits, cats, guinea pigs, dogs and rats. The most convenient bone for this purpose is the tibia which in rabbits and smaller animals can be pierced without difficulty by needles varying in proportion to the rodents' size. In larger animals, i.e., cats and dogs, the author employed special trocars or metal cannulae, which were inserted tightly into apertures bored through the bones with a fine drill or, more conveniently, with a sharp awl. The results obtained with epinephrine and also with ephedrine, a closely related drug, may be conveniently described under two headings, namely, experiments with aqueous solutions and experiments with oil suspensions.

Experiments with aqueous solutions. Injections of aqueous solutions of epinephrine into medullar cavities of the long bones of various animals produced unexpected results. The pharmacodynamie effects of such injections are hardly distinguishable from those following intravenous administration of similar solutions of the suprarenal medulla hormone. There is an immediate sharp rise in blood pressure of short duration followed by a rapid fall to below normal level and then a gradual return to normal level. As the subjoined illustration indicates, epinephrine in aqueous solutions is absorbed almost as rapidly after intraosseous injection as after administration by vein (fig. 1).

Subcutaneous and intramuscular injections of aqueous solutions of epinephrine

produced little or no rise in blood pressure of ordinary laboratory animals although clinical practitioners by such means have effected bronchodilatation in cases of bronchial asthma. More recently, certain clinicians using suspensions of epinephrine in oil in the treatment of bronchial spasm have claimed that their effect lasts longer than that produced by aqueous solutions of the hormone (4). While that is true with regard to the effect of epinephrine on the bronchi, blood pressure experiments on cats, rabbits and dogs have revealed little, if any, pressor effect on the blood pressure curve after hypodermic or intramuscular njection of either aqueous or oil solutions of epinephrine.

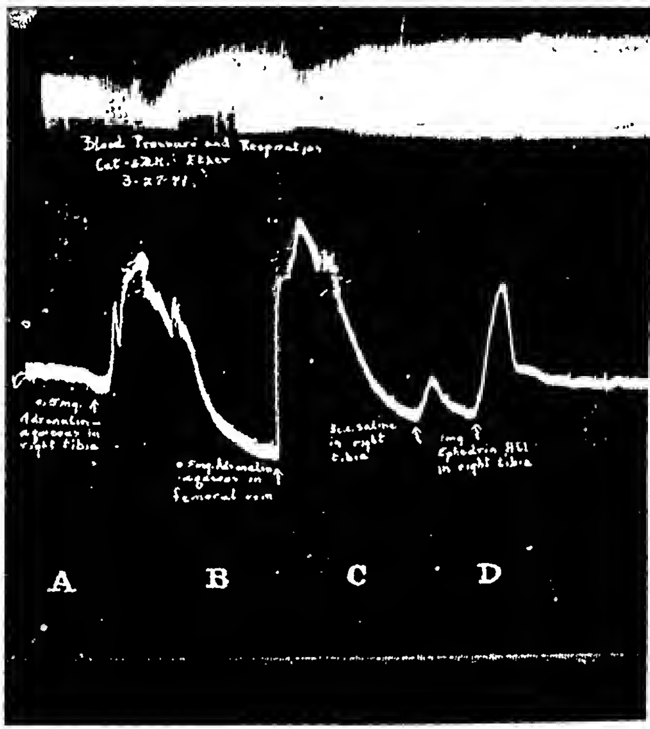

Fig. 1. Blood pressure and respiration of cat weighing 3.2 kilos under ether.
A. Injection of 0.5 mgm. of aqueous adrenalin in right tibia.
B. Injection of 0.5 mgm. of aqueous adrenalin in femoral vein.
C. Injection of 3 cc. of physiological NaCl solution in right tibia.
D. Injection of 1 mgm. of aqueous solution of ephedrine HCl in right tibia.

Experiments with oil suspensions. Very different results from those obtained with aqueous solutions were derived from experiments in which suspensions of epinephrine in oil were introduced into the medullar cavities of the long bones. Slow injections of a small quantity of adrenalin suspension, varying from 0.1 to 1.0 cc., depending on the size of the animals, were followed promptly by a sharp rise of the blood pressure to a moderate height, due undoubtedly to direct absorption of some of the oil suspension into the neighboring vessels. This rise in blood pressure is only half and sometimes less than half that noted after intraosseous injections of aqueous solutions, indicating that but a small amount of the suspension had been absorbed into the neighboring vessels.

On the other hand, the rise in blood pressure was not succeeded by the rapid return to normal level noted after both intravenous and intraosseous administration of aqueous epinephrine solutions. On the contrary, the pressor effect of epinephrine continued to be exerted for some time after its injection and in some cases lasted for as long as an hour (fig. 2). This remarkable sustained pressor effect of injections of epinephrine in oil into the medullar cavities is of special interest in connection with two problems immediately suggesting themselves to the physiologist. What is the explanation of this peculiar sustained effect and what is the incidence of oil embolism in such experiments?

In an endeavor to solve the second problem, the writer carried out a large number of control experiments with pharmacologically inert fixed oils, i.e., olive oil, peanut oil, peach kernel oil, sesame oil, cottonseed oil and mineral oil. Intramedullar injection of small quantities of such oils in the long bones of all the animals studied was usually harmless. Dangerous oil embolism occurred but rarely and deaths from such experiments with the fixed oils were exceptional

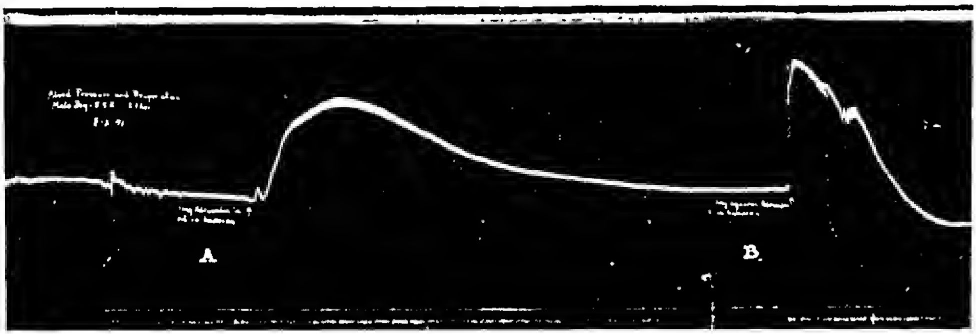

Fig. 2. Blood pressure and respiration of male dog, weighing 5.5 kilos, under ether.
A. Injection of 1 mgm. of adrenalin in oil in right humerus.
B. Injection of 1 mgm. of aqueous adrenalin in left humerus.

although their intravenous injection was usually fraught with great danger. It appears that the injection of such heavy or fixed oils into the medullar cavities is followed by but very slow absorption of the oil into the circulation, possibly in a more or less emulsified state. The prolonged action of epinephrine in maintaining a high blood pressure level in such experimental animals may be explained in the same way. These oil solutions of epinephrine apparently remain for considerable periods in the medullar cavities, which act as reservoirs for a drug that is slowly liberated and dispensed by the oil and passed into the circulating blood, where its pressor effect is maintained for a long time.

The comparatively innocuous effects of the fixed oils employed in the control experiments must not be confused with the effects of the so-called essential or volatile oils, which differ widely from the fixed oils in chemical composition, physical properties and physiological effects. All these essential oils, the writer has found, are rapidly absorbed through the skin and mucous membranes of small and large animals, producing toxic effects (5, 6). A similar action is rapidly exerted by the essential oils after their injection into the bone marrow.

The pharmacological or toxicological picture obtained with all of them is characteristic. There is a primary stage of excitement and agitation, followed by a depression and paralysis of the central nervous system and, finally, by coma, convulsions and death if sufficiently toxic doses of the oils be employed.

COMMENT. As compared with that following intravenous injection of aqueous solutions of epinephrine, the rapidity of absorption of such solution from the bone marrow is of special interest. Experiments reveal that aqueous solutions of adrenalin thus injected are absorbed almost as rapidly as when administered by vein. This phenomenon, observed by the writer after administration of aqueous solutions of all kinds of drugs, is confirmed by the enthusiastic reports of hematologists with regard to effective transfusion of blood plasma and whole blood in human beings. The curious findings obtained with intraosseous injections of epinephrine solutions *in oil* are of primary interest to the experimental biologist. Even though the writer's studies with intraosseous injection of fixed oils revealed that but a few cases of fatal embolism or gross pathological lesions are produced by such a procedure, it would be hazardous to draw hasty conclusions of a therapeutic nature from such experimentation and attempt to inject oil solutions of active principles into the medullar cavities of human beings before a great deal of further investigation had been completed with regard to the anatomy, physiology and pathology of the human osseous, vascular and hematopoietic systems as compared with those of lower animals. The cautious practitioner will always be mindful of the difficulties connected with the translation of observations on animals into clinical potentialities. The findings obtained with epinephrine, of course, are of great interest in connection with a study of certain phases of shock, and it is the writer's hope that they will stimulate further research along this line by both laboratory and clinical investigators.

SUMMARY

1. Intramuscular and hypodermic injections of both aqueous and oil solutions of epinephrine exert little or no effect on blood pressure of experimental animals.

2. Intraosseous injections of aqueous solutions of epinephrine are followed by immediate absorption of the hormone, as indicated by a prompt rise in blood pressure succeeded by a rapid fall to below normal level, effects which are not distinguishable from those following intravenous injection of such solutions.

3. Intramedullar injection of epinephrine suspensions in oil is followed by a prompt rise in blood pressure to a moderate level, long sustained, the maintenance of the pressure for a long time at that height indicating the slow and continuous absorption of this drug from the bone cavity.

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THE INITIATION OF IMPULSES IN CARDIAC MUSCLE

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Although the process of the initiation of impulses in the vertebrate heart has engaged the interest of physiologists for a long time, only few attempts have been made to study this problem directly and with negative results (1, 7). However, Arvanitaki (3), although working on entirely different material, the snail's heart, observed weak potential changes preceding the discharge of impulses. These results agree in their principal aspects with those described below for the vertebrate heart and with previous observations on visceral smooth muscles (6). In all these muscles weak non-conducted potentials initiate the spontaneous impulses. These local potentials, which resemble those of partly decalcified and spontaneously discharging nerve and skeletal muscle fibers (2, 3, 4), must be considered the basis underlying automaticity and will be called prepotentials.

A particularly interesting phenomenon are potential oscillations which, under certain conditions, initiate and follow impulses. Although these local potentials occur only in injured muscle they deserve attention because they explain some peculiar rhythmic phenomena previously described in isolated cardiac muscle. Furthermore, the regularity of the oscillations suggests that they express an important property of the tissue.

Methods. Strips from different parts of the heart of large turtles, rabbits, cats and dogs were used in most experiments. Monophasic potentials were obtained, as described previously for intestinal muscle (6), by forcing one end of a preparation inside a narrow glass tube and by attaching one of the leads to the part of the muscle thus inactivated. Diphasic artifacts were not always completely excluded by this technique but the potentials usually remained constant for several hours. A Toennies d. c. amplifier was used. Records were made either by a mechanical recorder writing on a smoked drum or by an oscillograph. It should be emphasized that the maximal height of the conducted potentials is not correctly indicated by the records because the impulses are too large (usually 5 to 10 mVolt) as compared with the local potentials and are, therefore, cut off by the amplifier.

Results. A. *Prepotentials.* To determine the processes which precede the discharge of an impulse, potentials were led off monophasically from the pacemaker. The precise origin of the impulses in a spontaneously beating muscle strip was found by placing the leads close together and by determining the point where the direction of the deflection reverses itself if the leads are successively shifted in one direction along the preparation. The correctness of this procedure, previously applied to the ureter, was confirmed by the fact that the location of the pacemaker so determined agreed with the region where the first

visible movements occurred when the preparation was removed from a refrigerator and was gradually warming up.

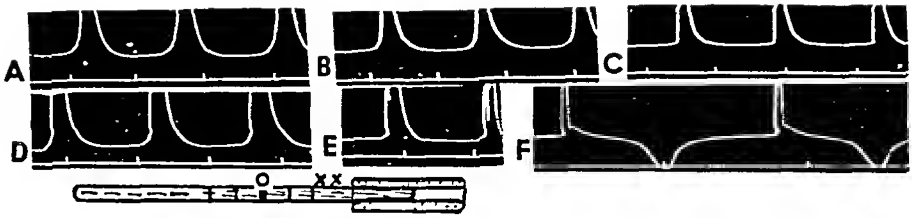
While the muscle shows normal rhythmicity each impulse is preceded by a period of gradually rising negativity. The significance of the local potentials is still more strikingly demonstrated in preparations in which the discharge is *induced by very regular potential oscillations.* These two phenomena will be described separately.

1. *Local potentials during normal rhythmicity.* If the active lead was placed on the origin of the impulses, potentials as shown in fig. 1, 2, 3A, 4A, 5A were usually obtained. A gradual rise in negativity preceded each impulse. That this phase of the action potential, amounting to 0.2 mVolt or less, is not due to the conduction of the impulse is shown by the fact that the ascent of the curve is sharp if both leads are on intact tissue and at some distance from the pacemaker (fig. 1F).

The phenomenon is, in many cases at least, partly due to a diphasic artifact or the disappearance of a positive after-potential (p. 7). However, it is significant that the rise in negativity at the pacemaker becomes progressively faster just before the discharge, whereas it would be expected to level off if it were entirely due to an after-effect of the previous impulse. The last phase of the potential change may, therefore, be interpreted as a prepotential.

This interpretation is confirmed by the observation that the size of the potential decreases on both sides of the pacemaker as shown in figure 1 and previously demonstrated for the ureter. In the turtle the prepotentials can be observed in the whole sinus region and their magnitude may be nearly the same over a distance of about 5 mm. This result agrees with the fact that the whole sinus possesses a considerable degree of automaticity and that, apparently, the precise origin of the impulses is determined by small quantitative differences between different regions. Sometimes also the pacemaker is moving back and forth along a preparation over a distance of two or more millimeters. These variations can easily be detected by placing the two leads close together in the region of the pacemaker. In such preparations, as would be expected, the size of the prepotentials fluctuates considerably.

Cardiac muscle is much less favorable for these studies than the ureter because the shortness of the intervals between beats makes it difficult to distinguish prepotentials from diphasic artifacts and after-potentials. The determination of the magnitude of the prepotentials in various regions gave convincing results only in about half the preparations used. In some preparations the potentials were too small to permit good comparisons. A more serious difficulty was found in the presence of an initial positive deflection in certain regions of the muscle, making the interpretation of the records uncertain. Furthermore, the prepotentials could not be detected in spontaneously beating preparations from the frog's ventricle nor in strips from the ventricle of the turtle in which activity had been induced by application of pure NaCl solution or Ringer's solution containing $BaCl_2$. These negative findings can be explained by assuming that in preparations with a low degree of automaticity the local potentials



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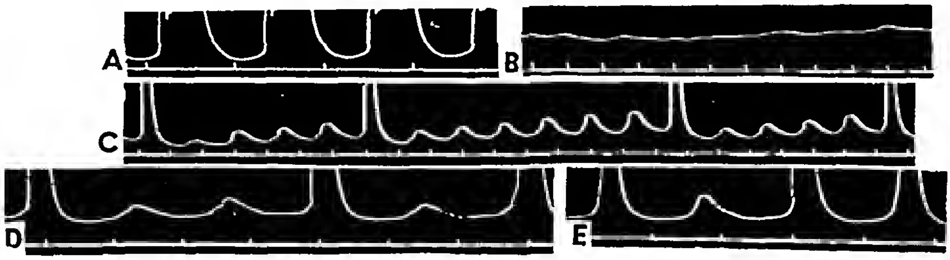

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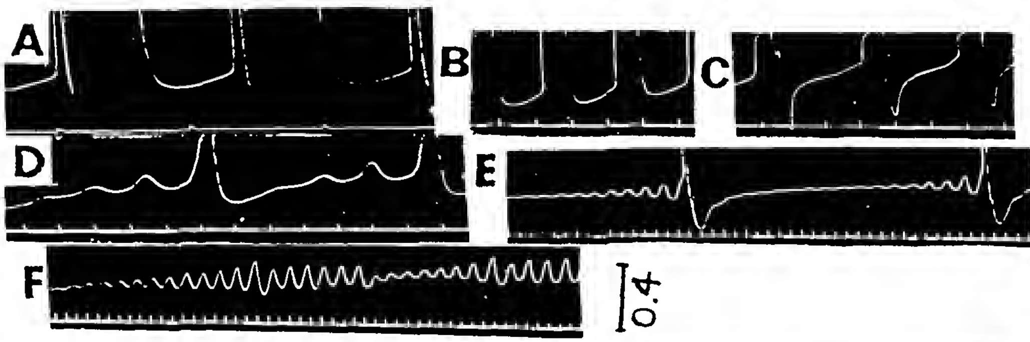

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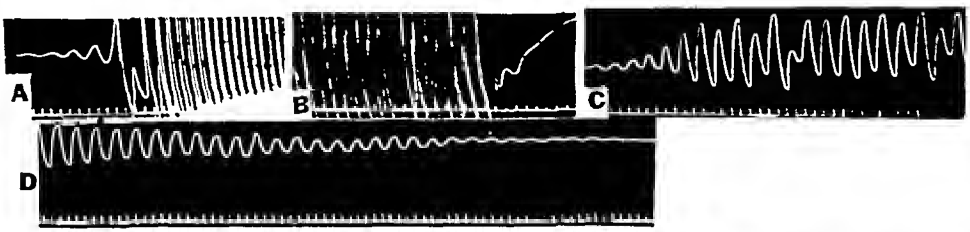

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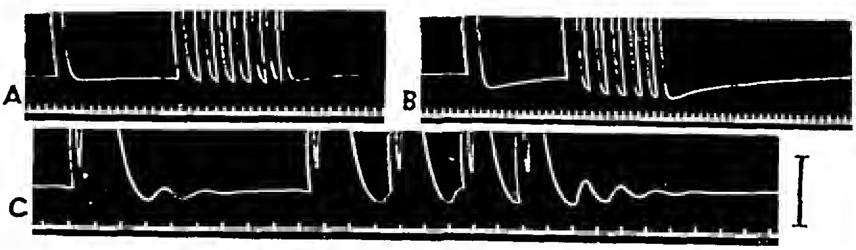

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Figs. 1–7

are restricted to small regions of the tissue or that they are weak and cause a discharge only if the excitability of the tissue is abnormally high.

More direct evidence for the existence of prepotentials was found in experiments where the muscle was depressed by injury or by acetylcholine. In the experiment illustrated in figure 2 a strip from the right auricle of the dog which had been dissected one day previously was used. It was at first quiescent and showed only slight and irregular electric activity. After a drop of adrenaline 1:100,000 was applied to one lead, the treated region began beating and potentials as shown in A were obtained. The discharge later ceased abruptly while slow rhythmic potentials continued (B). A comparison of these potentials with the prepotentials clearly shows that the gradual rise in negativity before each discharge is a phenomenon independent of the conducted impulses.

The local potentials can be separated from conducted impulses also by the application of drugs. Strips from the sinus venosus of the turtle and from the right auricle of mammals were irrigated with acetylcholine iodide 1:20,000 or β-methyl-acetylcholine (mecholyl) 1:400,000 and the recovery from the action of the drug was studied. All visible activity stopped but slow potential waves

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Fig. 1. Prepotentials at pacemaker and at various distances from it. Preparation strip from sinus venosus of turtle. Below diagram of arrangement of the leads, *O*, origin of impulses, *X* location of leads in record *F*. Monophasic potentials obtained by S tube around right end of muscle. A: active lead at *O*. B: 2 mm, C: 4 mm. to right, D: 2 mm, E: 4 mm to left, F: diphasic. Time: seconds. Temp.: 22°.

Fig. 2. Potentials of a strip from the right auricle of the dog. Both leads on intact tissue. Activity was initiated by applying adrenaline $1:10^6$ to the region of one of the leads. In B sudden cessation of discharge with continuation of local potentials. Calibration 0.5 mVolt. Time: seconds. Temp.: 32°.

Fig. 3. Action of acetylcholine on the sinus venosus of the turtle. Monophasic. A, normal beat. B, after application of acetylcholine 1:20,000, only local potential at previous frequency. C, partial recovery, fully conducted impulse only every second local potential wave. The drug caused restoration of conduction inside glass tube, producing diphasic potentials. Time: $\frac{1}{5}$ second.

Fig. 4. Action of mecholyl on strip of sinus venosus of turtle. After A was recorded mecholyl 1:400,000 was applied. In B somewhat irregular, weak local potentials. C, D, E, stages of the recovery from the action of the drug. Note missed beats of pacemaker. Time: seconds.

Fig. 5. Oscillatory prepotentials from strip of sinus venosus of turtle produced by an excess of K ions. A, normal muscle. B, C, D, after irrigation with a mixture of Ringer's and 1 per cent KCl in the proportions 1:10, 2:10, 3:10 respectively. Without additional treatment in E further slowing of beat and increased number of oscillations, in F cessation of discharge while local oscillations persist. Temp.: 26°. Time: seconds.

Fig. 6. Luciani group from strip of right auricle of the cat showing oscillatory prepotentials. A, beginning, B, end of a group. Second beat missed as in all groups from this preparation. C, local oscillations without discharge shortly before D, final cessation of electric activity was recorded. In C and D amplification twice that in A and B. Temp.: 29°. Time: seconds.

Fig. 7. After-potentials of strip from turtle ventricle. Monophasic. Response elicited by electric shocks. A, Ringer's solution; B, after irrigation with isotonic NaCl solution; C, after irrigation with a mixture of equal parts of Ringer's and isotonic $CaCl_2$. Temp.: 22°. Time: seconds. Calibration: one mVolt.

were usually present. In many details the results were variable. In eight of the twenty preparations used the potential waves had the same frequency as the normal beat (fig. 3). Their size gradually increased until the discharge of impulses was resumed. At first the intervals between beats were long, as long as ten times the normal duration of a cycle, because the muscle missed some of the beats of the pacemaker. In the other preparations the activity of the pacemaker was at first rather irregular but returned to the previous frequency as soon as some impulses were discharged (fig. 4), or the frequency of impulses at first was definitely smaller than normal, though never less than half. It appears that an appreciable slowing of the rate by acetylcholine is chiefly caused by a failure of the muscle to respond regularly to the rhythmic local potentials and only to a minor extent by a slowing of the intrinsic rhythmicity.

The potentials during the recovery from the drug action often are very complex. This is, at least partly, due to the appearance of impulses which are conducted for only short distances (p. 14) and to some inco-ordination between different parts of the preparation. Sometimes another, independent, pacemaker comes into play temporarily as shown by the fact that the first conducted impulses are discharged without any relation to the beat of the previous pacemaker whose activity is being recorded.

2. *Oscillatory prepotentials.* In depressed cardiac muscle the impulses often are preceded by a series of potential oscillations of gradually increasing magnitude like those previously described for the ureter and other spontaneously discharging tissues (3, 6). Discharges always occur near a negative crest of one of the potential oscillations. The phenomenon could be produced by a variety of agents, by an excess of K or Ca ions or by mecholyl, or it occurred in preparations kept in the refrigerator overnight, but no treatment was found which invariably induced oscillatory prepotentials.

It is remarkable that, while the frequency of impulses may be slowed down many times, the intrinsic rhythmicity of the pacemaker can be modified only moderately. Thus in figure 5 moderate amounts of K-ions slowed down the intervals between beats (B), but as the intervals became longer (C) potential waves appeared between the beats at a frequency close to that of the normal beat and these rhythmic variations continued at high K ion concentrations with unchanged frequency while no impulses were discharged and until all signs of activity had disappeared (D).

In preparations with oscillatory prepotentials impulses often are discharged in groups because several waves in succession initiate an impulse. In the preparation from which figure 6 was obtained, 14 almost identical groups, each consisting of 40 to 60 beats, were recorded. During the intervening period of about 2 minutes no rhythmic activity could be detected. At the end of the series there were groups of oscillations without any discharge (fig. 6C). The rhythmicity of the muscle remained perfectly regular until all signs of spontaneous activity stopped (D). The grouping of the impulses into bursts has previously been described under the name of "Luciani periods" and was observed under a variety of conditions injurious to the muscle (cf. 11). An analogous phenom-

enon, also characterized by oscillatory prepotentials, was found in the ureter under similar conditions.

B. *After-potentials.* The after-potentials of cardiac muscle will be described here because, like the prepotentials, they may initiate impulses and are important for the understanding of certain rhythmic phenomena.

The possibility of a diphasic artifact makes the interpretation of these potentials uncertain during the first second, but the later phases undoubtedly represent a separate phenomenon.

In quiescent, fresh strips from the turtle's heart each response is followed by a weak positive after-potential lasting for about 10 seconds. It is increased after a short series of responses (fig. 7) but after prolonged stimulation it becomes smaller again or it may even be replaced by a negative after-potential. The positive after-potential is furthermore increased by irrigation with calcium free Ringer's solution or isotonic NaCl solution (fig. 7B). After repeated irrigations the preparations often beat spontaneously at a fairly rapid rate so that the after-

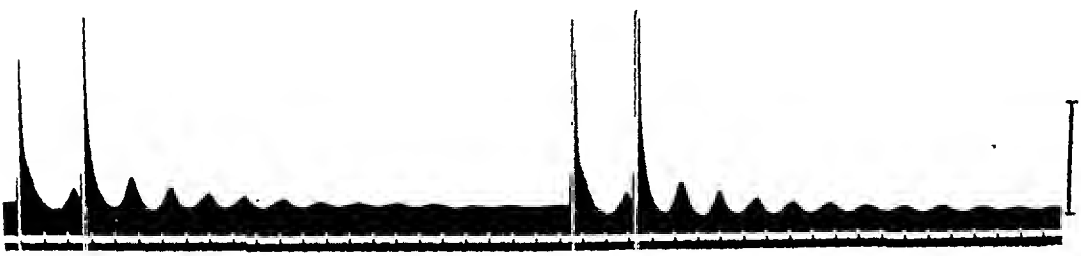


Fig. 8. Oscillatory after-potentials from a strip of the right auricle of the rabbit produced by application of a mixture of equal parts of Ringer's and isotonic $CaCl_2$. Monophasic. Groups of two impulses, discharged independently of the oscillations. Recorded with oscillograph. Time: $\frac{1}{5}$ second. Temp.: 34°. Calibration: 0.5 mVolt.

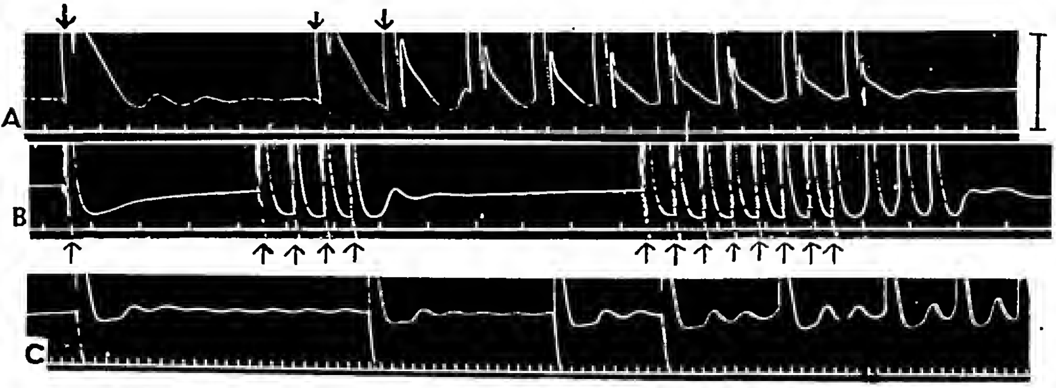
potentials cannot be studied. In preparations in which this does not occur the positive after-potential gradually becomes smaller again and finally a single slow negative wave appears instead.

In analogy with nerve, one might expect that the positive after-potential is associated with subnormality and it is particularly suggestive to assume that its presence causes the inhibition which follows a series of extrasystoles in the auricle as well as ventricle (Erlanger and Hirschfelder, 8; for later work, cf. 11). However, in preliminary observations no correlation between the duration of the positive after-potential and the duration of the inhibition could be observed.

Muscles which are studied one day after dissection often show oscillatory after-potentials consisting of waves of gradually decreasing magnitude resembling damped oscillations. This phenomenon can be produced most easily in strips from the turtle heart by treatment with a solution containing a large excess of Ca-ions (fig. 7C). The oscillations are present only in the region treated with the solution. Following a short series of responses they are larger than after a single discharge, but the amplitude declines after more than about ten responses. An excess of calcium ions sometimes produces this type of after-

potential also in preparations from mammalian hearts (fig. 8). The effects of the solutions used are completely reversible.

Adrenaline added to the solutions (1:10[6]) increases, usually about doubles, the magnitude of the oscillations in the auricle and ventricle. Acetylcholine



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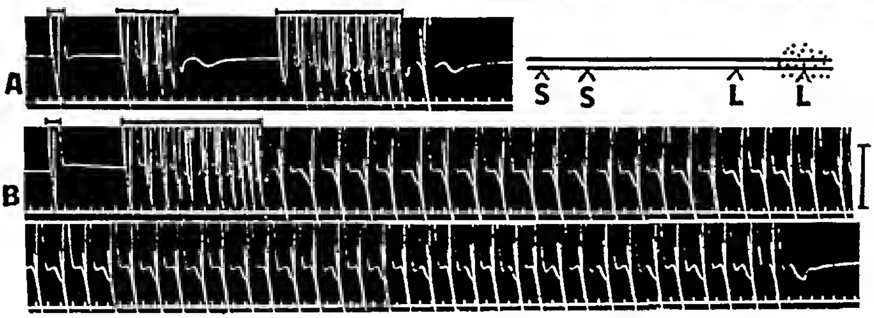

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Fig. 9. After-discharge produced by oscillatory after-potentials. Stimuli are indicated by arrows. All the other impulses induced by oscillations. A, strip from turtle ventricle treated with equal parts of Ringer's and isotonic $CaCl_2$. Temp.: 22°. Calibration one mVolt. B, strip from right auricle of dog 24 hours after dissection. Monophasic. Temp.: 32°. C, shift in the origin of impulses as a result of the appearance of oscillations. Strip from the sinus venosus of the turtle treated with mixture of 2 parts isotonic $CaCl_2$ and one part Ringer's. All impulses discharged spontaneously. Discharge ceased about 3 minutes later. After a period of rest, during which oscillations almost disappeared, the same sequence was recorded three times. Temp.: 19°. Time: seconds.

Fig. 10. After-discharge produced by oscillatory after-potentials, showing that it originates in region with oscillations. Strip from ventricle of turtle. Diphasic potentials. Arrangement of electrodes shown in diagram. S, stimulating electrodes; L, leads for amplifier. Region of right lead was treated with a mixture of equal parts of Ringer's and isotonic $CaCl_2$. The initial impulses (indicated by horizontal line above), beginning with an upstroke, were elicited by electric shocks. The impulses of the after-discharge begin with a slow down-stroke which is the first wave of the after-potential. In the upper record the after-discharge had only one impulse, in the lower record from the same preparation, a longer period of stimulation was followed by an unusually long after-discharge. Calibration: 1 mVolt. Time: seconds.

and mecholyl strongly depress the oscillations in the auricle but have no effect on the ventricle, an observation which is in line with the absence of other inhibitory effects of acetylcholine on the ventricle. It is remarkable that the

drugs studied may increase or decrease the magnitude of the oscillations but do not change their frequency.

The oscillatory after-potentials are particularly interesting because they may cause the discharge of impulses. In preparations with oscillatory after-potentials a short series of responses, rarely also a single response, may be followed by additional impulses which I shall call the after-discharge (fig. 9A, B). These impulses arise from the negative phases of the after-potential and are fired off if the latter reach threshold value. The tendency to give after-discharge is greatest under conditions where the after-potentials are largest, following a short series of responses and under the influence of adrenaline.

That the impulses of the after-discharge do not originate at the stimulating electrode is shown in the experiment illustrated in figure 10. Diphasic potentials were recorded and the region of the distal lead alone was treated with a solution containing an excess of calcium ions. The impulses of the after-discharge were conducted back toward the stimulating electrodes and evidently originated in the region treated with the solution. The after-discharge usually consists of one or a few impulses but may also last for several minutes. The termination of the after-discharge can be explained by the gradual decrease in the size of the oscillations during a long series of responses.

The presence of oscillatory after-potentials may lead to peculiar rhythmic phenomena. In the experiment illustrated in figure 9C a preparation from the sinus venosus of the turtle was treated with a large excess of calcium ions. The beat became intermittent and each series of impulses was introduced by an accelerating series of beats originating close to the distal lead. The oscillations induced by these spontaneous beats increased after each discharge until they were so large that they determined the rhythm of the beat and the pacemaker shifted suddenly to the region of the first lead.

After-discharge consisting of one single impulse had previously been observed by Schmitt and Erlanger (12) who explained the phenomenon as due to the reentry of an impulse and called it opisthodromic conduction. Ashman and Hafkesbring (5) observed after-discharges of longer duration and attributed them to the presence of a phase of supernormal excitability.

Recently Harris and Moe (19) described rapid oscillations produced by constant current of high intensity, which they consider analogous to the oscillations observed by Arvanitaki in nerve fibers. However, the conditions under which the observations were made and the high frequency of the oscillations make it doubtful that they are similar to the decrementing and incrementing local potentials described here.

Discussion. It appears that there are two types of rhythmic local potentials as had previously been observed in the ureter. In the first type, which is responsible for the initiation of the normal beat and can most conveniently be studied after the application of acetylcholine, repolarization follows the phase of depolarization rather abruptly, and the potential waves sometimes continue for appreciable periods without great changes in magnitude (fig. 2B, 3B). Their frequency is influenced, in some cases, by acetylcholine and by adrenaline,

and it varies considerably between different preparations. The second type, found only in injured muscle, is distinguished by its great regularity and it resembles in its time relations oscillations of simple physical systems. Their magnitude usually increases and decreases in a regular manner and, in the turtle heart, their frequency is surprisingly constant and unaffected by acetylcholine, adrenaline, or even by extreme variations of the ionic composition of the medium. How the first type of potentials may change over into the second type is illustrated in figure 5.

The ascent and descent of the potential waves, as long as they are weak, are usually roughly symmetrical. However, as their magnitude approaches threshold the ascent becomes progressively steeper as compared with the descent. This effect may be due to an additional non-propagated response which is, perhaps, analogous to the local response in non-medullated nerve fibers produced by electric shocks of near threshold strength (10). In fully excitable muscles the propagated impulses arise quite abruptly from the local response. However, in muscles which have been injured or treated with high concentrations of acetylcholine responses of many different sizes, intermediate between local responses and fully conducted impulses, may be found. Probably these responses are caused by impulses which are conducted only for short distances, and, therefore, are comparable to the responses of depressed nerve fibers where likewise intermediate stages between local responses and fully conducted impulses occur (10).

That there is no essential difference between the oscillatory pre- and after-potentials is suggested by the observation that both can initiate impulses and by the fact that their frequency is the same. Furthermore, in preparations in which the prepotentials are oscillatory the after-potentials have the same character and, in fact, one may consider the after-discharge as a Luciani period induced by electric stimulation. The difference between preparations showing this phenomenon, like strips from the ventricle of the turtle and muscles which often give spontaneous Luciani periods, for example the sinus venosus, lies merely in the different degree of automaticity.

The magnitude of the oscillations of the after-potential may diminish rapidly so that only one or two waves can be seen or they may decrease so slowly that as many as thirty oscillations can be counted. To describe these differences one may speak of different degrees of damping (cf. 3, 4). It is significant that the damping is smallest in preparations with automaticity (fig. 8, 9C), like those of the sinus venosus, and largest in preparations which never discharge spontaneously, like those from the turtle's ventricle. Prepotentials can also be considered in this connection and they can be characterized by negative damping. There appear to be all transitions between these potentials and the highly damped after-potentials of ventricular muscle.

It is apparent that many rhythmic phenomena in cardiac muscle cannot be understood if only conducted impulses are recorded. It is quite possible, therefore, that the local, graded activity which may take place in the "resting" state offers the key to the understanding of a variety of disturbances of the rhyth-

micity of the heart. The oscillatory after-potentials, for instance, provide a simple explanation for coupled extrasystoles and paroxysmal tachycardia. Unfortunately the study of these weak and localized potentials in the heart in situ presents great technical difficulties.

SUMMARY

The action potentials of isolated cardiac muscle were recorded in an attempt to detect the processes which initiate the beats and which are responsible for the rhythmicity of heart action. Confirming earlier work on other muscles with automaticity it was found that spontaneous impulses are initiated by weak local potentials which are present in a large part of the muscular tissue but which are strongest near the origin of the impulses. A phase of gradually rising negativity precedes each impulse in muscles with normal rhythmicity, but in injured muscles there may be instead regular potential oscillations of gradually increasing magnitude. These oscillations give rise to the phenomenon of the Luciani periods if several waves in succession cause the discharge of impulses.

Each impulse is followed by an after-potential which is normally positive but which is oscillatory under certain abnormal conditions. The oscillations of the after-potential may give rise to the discharge of further impulses like those of the prepotentials. Acetylcholine diminishes the magnitude of the oscillations in the auricle and sinus venosus, but not in the ventricle; adrenaline increases their magnitude. None of the drugs alters their frequency.

It may be expected that a consideration of the local potentials will be helpful in the understanding of certain types of abnormal rhythmicity of the heart.

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THE ELECTRICAL ACTIVITY OF A THALAMOCORTICAL RELAY SYSTEM

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From time to time in the course of experiments dealing with cerebral pathways and thalamocortical relationships, it was observed that single stimuli applied to peripheral nerves or to ascending sensory pathways produced a repetitive series of cortical potentials in addition to the usual sensory response (Morison, Dempsey and Morison, 1941a), and that these repetitive potentials were sharply localized within the cortical area receiving sensory projection fibers (cf. Adrian, 1941). The "repetitive sensory response" was grossly similar, except for its localization, to the spontaneous bursts of cortical activity characteristic of nembutal anesthesia and to the bursts of activity set up by the recruitment response (Dempsey and Morison, 1942a). Because of these similarities, it was frequently difficult to determine whether the response to a given stimulus resulted from activity in medial thalamic mechanisms (Morison and Dempsey, 1942) or to activity in other circuits whose localization was unknown. Similarly, repetitive stimulation frequently brought about a successive increase in the evoked potentials reminiscent of the recruiting response consequent on stimulation of intralaminar thalamic structures (Dempsey and Morison, 1942a). Striking differences in latency and anatomical localization, however, suggested that an attempt to separate these various phenomena one from another by as many criteria as possible might illuminate the problem of thalamocortical interaction and consequently facilitate the analysis of spontaneous cortical activity. It was therefore decided to extend these investigations in order to determine the characteristics of the various effects for comparison with those of other cerebral systems.

Material and methods. Cats, anesthetized with nembutal (0.65 cc./kgm), were used. Condenser discharge stimuli were led to the animal through a transformer and a Wagner ground for controlling the shock artifact. Cortical responses were picked up by bipolar silver or steel electrodes applied either on the surface of the pia or from the pia to a deep electrode inserted about 2 mm. into the cortical substance. The usual procedure was to explore the cortex with the surface bipolar electrodes until the point yielding maximal potentials to peripheral nerve stimulation was reached. At this point the deep electrode was inserted into the cortex, and subsequent observations were made so that an upward deflection signified negativity at the surface electrode. Bipolar steel wires, insulated except at the tips and having a separation distance of approximately one-half millimeter, were inserted into the brain by means of a stereotaxic instrument (Morison, Dempsey and Morison, 1941b) and utilized either for recording or stimulating. After suitable amplification, the potentials were re-

corded by a 5-channel Grass inkwriter, a DuBois oscillograph or a 2-channel cathode ray oscillograph.

At the end of each experiment the brain was removed and fixed in 10 per cent formalin. Serial frozen sections were prepared (Marshall, 1940) and stained with thionin. A direct positive print was prepared by projecting at 10 times magnification the sections containing the needle tracks made by the stimulating and recording electrodes. A permanent record was therefore provided of the electrode position corresponding to each electrical record taken during the experiment.

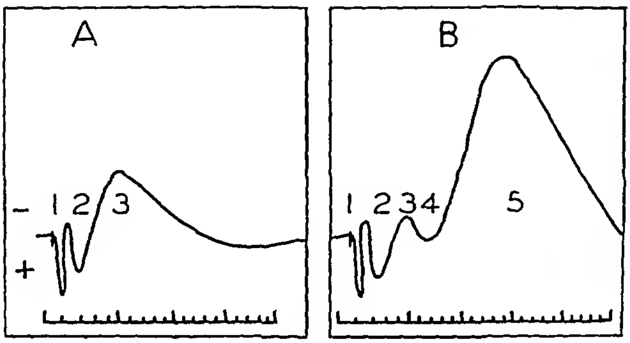

Fig. 1

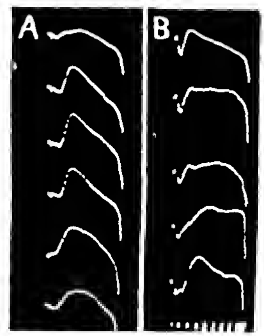

Fig. 2

Fig. 1. Tracings of cortical responses to stimulation of the internal capsule. The potentials were recorded by surface to deep electrodes, one pole on the pial surface and one inserted 2 mm. into the cortical substance. The electrodes were connected so that an upward deflection signifies negativity at the surface electrode. Time scale, 1 + 5 msec.

A. Response to single shock. Three components are present, the first two being positive and the third a negative deflection. This complex is referred to in the present paper as the "primary response."

B. The fourth response to a series of stimuli at a frequency of 8 per second. A fourth, positive and a fifth, negative deflection have appeared following the original three components. Components 4 and 5 increase dramatically in size during repetitive stimulation, and are referred to as the "augmenting response."

Fig. 2. Separation of thalamic regions yielding primary and augmenting responses.

A. Stimulus to medial part of *n. ventralis lateralis pars externa*. Pure augmenting effects are shown in the cortex.

B. Stimulus 1 mm. lateral to the position from which record A was obtained. Both primary and augmenting effects are present. Time signal, 250 cps.

Results. A. *Description of responses.* When single shocks were applied to the ventrolateral thalamus, cortical responses occurred in which three component deflections could be recognized: 1, a surface positive, spike-like deflection with a latency so short that it frequently merged with the stimulus artifact; 2, a positive deflection of 0.5 to 0.8 msec. latency which was followed by 3, a prolonged negative deflection.

Application of a short train of stimuli at a suitable frequency caused the appearance of two further components. These were: 4, a positive deflection whose latency was approximately the same as the negative peak of the third element; and 5, a second negative element. Figure 1 indicates diagrammatically the typical sequence, latency and duration of these components.

The diverse components of the wave complex reacted differently in various experimental conditions. The first element followed higher frequencies of stimulation than any of the others, and, although reduced in size, was still recognizable at stimulus frequencies of 400 per second. Next most resistant to high frequencies was the second component which remained at stimulus frequencies of around 100 per second. The third element usually was severely reduced at frequencies of 30 to 50 per second.

Stimulation with lower frequencies (optimal at 8–15 per sec.) was followed by the progressive development, after the first shock, of the 4th and 5th components (fig. 3, A and B). This progressive enhancement is a striking characteristic and has suggested the term "augmenting response." The first three components, on the other hand, showed no augmentation but only reduction on repetitive stimulation. They are referred to in the present paper as the "primary response" (fig. 3, C and D).

In favorable preparations, the primary potential, elicited by a single shock, was followed by a train of waves the frequency of which was similar to that of spontaneous bursts which ordinarily constitute the predominant cortical activity of the nembutalized cat (fig. 7A). This "repetitive sensory response" was not seen invariably (cf. Adrian, 1941), nor have the precise conditions necessary for its production been identified, though light anesthesia may be said to favor its occurrence.

B. *Anatomical localization.* The cortical area from which the augmented response could be recorded varied with the placement of the stimulating electrodes. Shocks just above threshold strength ordinarily produced primary responses localized to the primary divisions of the sensory cortex and augmented responses localized to these and the corresponding areas anterior and posterior. These observations indicate that whenever primary and augmented responses were both provoked, the cortical area yielding augmented responses was greater than that from which the primary response could be recorded.

In several experiments observations were made which suggest an anatomical separation of the systems responsible for the two types of effects. Thus in certain positions of the stimulating electrodes only primary responses were produced, while in others pure augmenting potentials were obtained (fig. 2A). In either location, application of stimuli of greater intensity was followed by the total wave complex, i.e., by both primary and augmenting responses (fig. 2B).

Results identical with those produced by thalamic stimulation were obtained, when the stimulating electrodes were placed in the internal capsule. Here, as from thalamic activation, primary responses were localized to smaller cortical regions than were the augmenting effects, and capsular areas yielding only primary or augmenting responses were found.

Stimulation of the medial lemniscus and sensory nerves with repetitive volleys also was followed by effects attributable to augmentation. The sensory nerve effects were less striking than were those of the lemniscus, thalamic nucleus and internal capsule. Nevertheless, in favorable preparations nerve activation produced clear augmenting responses. In the majority of the experiments, how-

ever, the voltage of the sensory potential complex did not increase appreciably, but changes in the wave form of the response occurred. The positive initial deflection broadened and the negative response was delayed (fig. 4). These changes have been interpreted as an addition of the augmenting effect to the shorter latency true primary response.

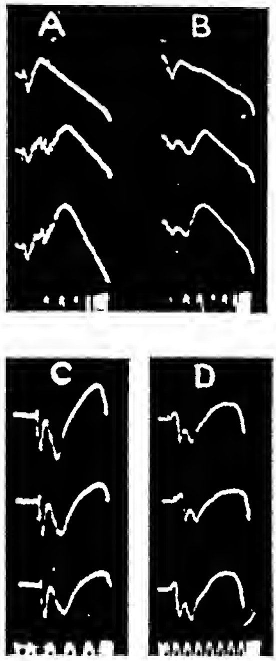

Fig. 3

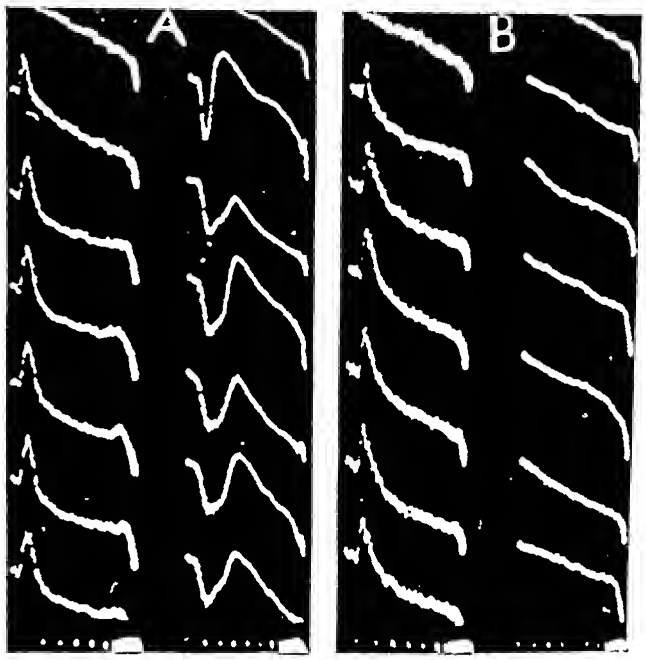

Fig. 4

Fig. 3. Primary and augmenting responses induced by sensory stimulation.

A. Surface to deep recording electrodes. Stimulation 8/sec. to internal capsule. Time signal, 200 cps. Both primary and augmenting responses are present. Compare with figure 1.

B. Same as A, but surface bipolar electrodes.

C. Stimulus to another position in the internal capsule. Only the primary response is present. Compare wave form with figure 1A. Time signal, 500 cps.

D. Stimulus to medial lemniscus. The wave form is similar to that seen after capsular stimulation, but the latency is longer. Time signal, 500 cps.

Fig. 4. Augmentation in thalamus and cortex on repeated stimulation of a sensory nerve. Simultaneous records: left, from *n. ventralis lateralis pars externa;* right, from radial cortical projection. Stimuli to radial nerve at 8 per second.

A. Note broadening of thalamic response. The duration of the positive cortical wave increases and the negative peak is shifted to the right. Time signals, 100 cps.

B. Same after decortication. The broadening of the thalamic response is not abolished. Time signals, 100 cps.

The repetitive sensory response was confined quite sharply to the area of the sensory cortex yielding primary potentials (cf. Adrian, 1941). In addition to its appearance in the sensory cortex, the repetitive response was recorded from electrodes placed in *n. ventralis lateralis pars externa* of the thalamus (fig. 5) and from the internal capsule. Repetitive potentials did not occur, however,

when recording electrodes were inserted into the medial lemniscus posterior to the lateral thalamic nuclei.

The repetitive cortical response has been evoked by single stimuli applied anywhere along the ascending sensory system. Indeed, it was more easily elicited by stimuli delivered to the medial lemniscus or thalamic nuclei than by activation of the peripheral nerve. It was frequently observed that preparations whose response to nerve stimulation was poor yielded excellent responses when stimulated either at the level of the thalamic synapse or the thalamic radiations.

It is apparent from a consideration of the characteristics of the primary response (elements 1, 2 and 3) and the augmented response (elements 4 and 5) that they react in various respects as two independent units. The differences in

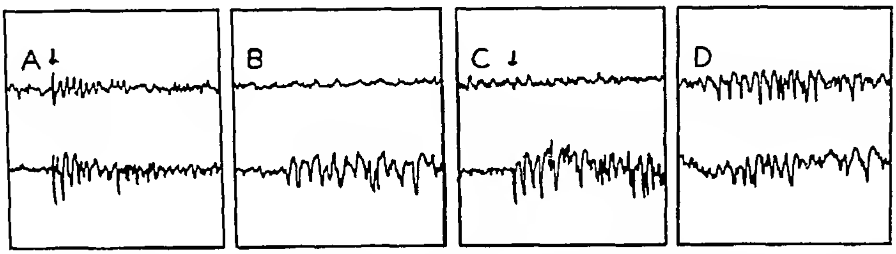

Fig. 5. Localization in thalamus of repetitive sensory response and burst activity. Upper records from thalamus; lower records from radial cortical projection. Arrows signal a single stimulus to the radial nerve. Paper speed 7.5 mm. per sec.

A. Thalamic electrodes in *n. ventralis lateralis pars externa*. Note immediate response to radial stimulus, followed by repetitive waves in cortex and thalamus.

B. Same recording position. The record is of a spontaneous cortical burst. Note absence of thalamic activity.

C. Thalamic electrodes 2 mm. medial and 1 mm. above the position from which records A and B were obtained. The immediate and repetitive sensory responses are not obtainable at this location.

D. Same recording position. Note activity in thalamus correlated with the spontaneous cortical burst.

behavior of these two units may be summarized as follows. 1. The augmenting response ordinarily requires previous facilitation for its appearance, while the primary response appears full sized after a single stimulus. 2. The primary response always has a shorter latency than that of the augmenting response. Consequently, capsular or thalamic stimulation results in clearly separable effects, while after sensory nerve stimulation the greater opportunities for temporal dispersion result in the augmenting response appearing as a distortion of the terminal phase of the primary response. 3. The primary response is localized to a smaller area in the cortex and thalamus than is the augmenting response, and for any given stimulus, areas can be found from which each response is recordable without the other.

C. *Interactions of thalamocortical potentials.* 1. *Repetitive, augmenting and primary sensory responses.* The various considerations outlined above indicate that the augmenting response represents a state of physiological activity different

from that indicated by the primary response. The alternative view, that mere recruitment of similar but slower elements constitutes the difference between them, would imply that no qualitative differences in behavior should occur. Consequently, experiments were performed in which the repetitive response was induced by application of a single shock delivered variously to the peripheral nerve, thalamic nucleus or thalamic radiations. At varying intervals after this first shock a second stimulus was applied to the peripheral nerve so that the

Fig. 6

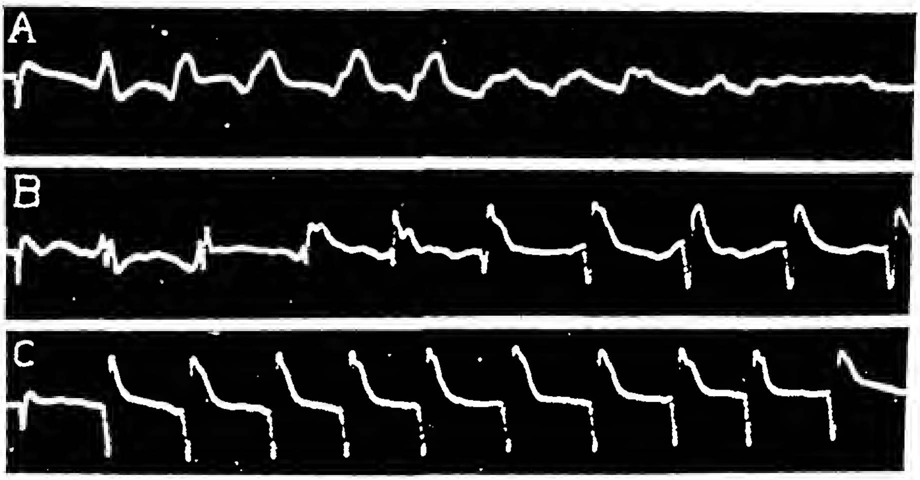

Fig. 7

Fig. 6. Superimposition of the sensory primary response upon the repetitive potential. Two shocks were delivered to the radial nerve timed so that the second fell upon the repetitive potential at varying phases. Record from radial cortical projection, surface to deep electrodes. Time signal, 10 cps.

Fig. 7. Interaction of the repetitive response with the augmenting sensory response. Radial cortical projection, surface to deep electrodes. Stimuli to the ventrolateral thalamic nucleus.

A. Single shock, showing immediate and repetitive responses. Note that the first five repetitive potentials are nearly equal and are followed by 3 or 4 smaller effects suggesting a stepwise gradation in size.

B. Stimuli at a frequency of 7 per sec. At this rate each succeeding stimulus falls upon the repetitive potentials, and augmentation does not occur until the repetitive effect is fatigued.

C. Stimuli at a frequency of 8 per sec. Each stimulus precedes the repetitive effect and immediate augmentation is produced.

primary response fell at varying phases upon the individual potentials of the repetitive response. The primary response induced by the second test shock was not blocked at any phase of the repetitive response (fig. 6). On the other hand, evidence was supplied by experiments such as that illustrated in figure 7B which shows that succeeding shocks when timed to fall during a repetitive potential failed to evoke augmented responses. Slightly shorter intervals between stimuli resulted at once in good augmentation but obliterated the repetitive response (fig. 7C). The conclusion seems inescapable that the augmenting and primary responses represent activity in separate neuronal systems since they interact dif-

erently with the repetitive effect. Similarly, the obliteration of either the augmenting by the repetitive potential or *vice versa* strongly suggests that they represent different states of activity in the same neuronal system.

2. *Repetitive sensory response and spontaneous activity.* The similarity in wave form and general appearance of the repetitive response and the generalized 8–12 per sec. spontaneous potentials which characterize nembutal anesthesia prompted an investigation of their interactions. The repetitive response was evoked at various phases of the spontaneous bursts. Stimuli delivered during a burst and timed so as to throw into phase the repetitive and spontaneous rhythms enhanced the potentials immediately following the shock. Moreover, when the

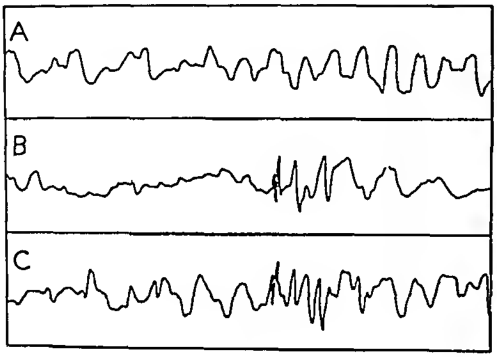

Fig. 8

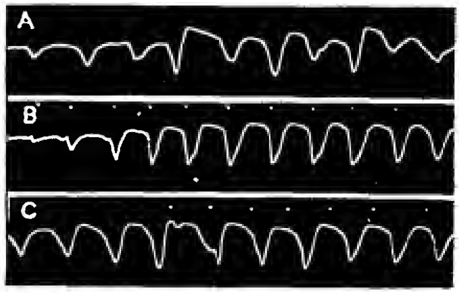

Fig. 9

Fig. 8. Interaction of repetitive and spontaneous burst effects. Radial cortical projection, surface to deep electrodes. Paper speed, 30 mm. per sec.

A. Spontaneous burst.

B. Immediate and repetitive effect of a single radial nerve stimulus.

C. The repetitive effect induced out of phase with the spontaneous potentials. Note the doubling of the cortical frequency.

Fig. 9. Superimposition of augmenting sensory response upon spontaneous burst potentials. Radial cortical projection. Surface to deep electrodes. Stimuli at a frequency of 10 per sec. signaled by dots.

A. Spontaneous burst.

B. Augmenting responses induced during an interval between bursts.

C. Augmenting responses induced during a burst of spontaneous potentials. Note the appearance of augmented responses when evoked during the spontaneous effects.

repetitive response was induced out of phase with the spontaneous burst, the individual repetitive potentials were interpolated between the spontaneous waves in such a way that the cortical rhythm suddenly doubled in frequency (fig. 8). Consequently, since two forms of activity can not simultaneously occupy a common pathway, it follows that the neuronal elements responsible for the burst and the repetitive effects are different.

Spontaneous activity other than the 8–12 per sec. rhythmic bursts can also be distinguished in the electrocorticogram. Varying amounts of random activity existed during the intervals between bursts (cf. Derbyshire, Rempel, Forbes and Lambert, 1936). This activity was more consistently recorded from electrodes placed upon the posterior sigmoid, marginal and ectosylvian gyri than from

other cortical areas. This characteristic has prompted the tentative name of "projection" activity which will be used throughout this paper.

The amount of this background projection activity ordinarily varied directly with the level of anesthesia, although in a few preparations large amounts of nembutal were required to suppress it. In such preparations, however, the activity was greatly reduced by decerebration (fig. 10). This fact indicates a difference between the projection and burst activities, since the latter was not depressed by section of the brain stem (fig. 10B). However, section of the thalamic radiations or removal of the entire thalamus abolished both projection and burst activity, leaving only the widely separated waves which have been associated with an extrathalamic system (Morison, Dempsey and Morison, 1941b).

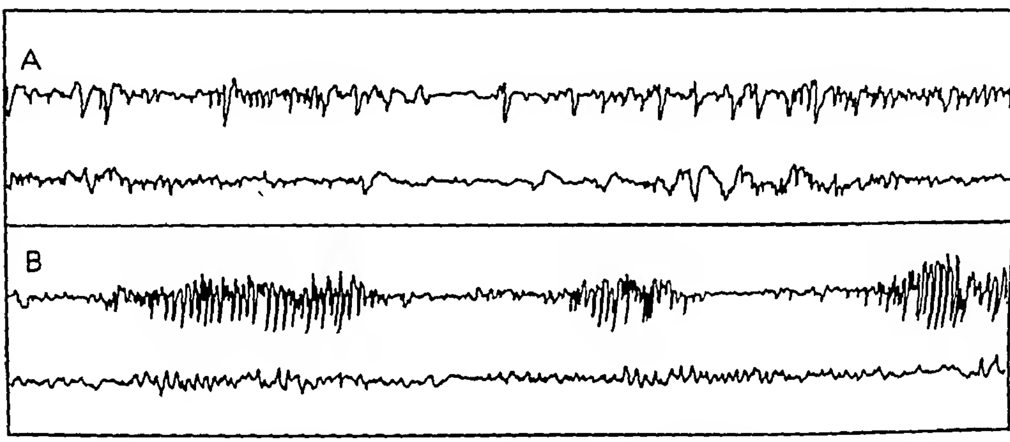

Fig. 10. Effect of decerebration upon spontaneous "projection" and "burst" activity. Upper record, radial cortical projection. Lower record, middle suprasylvian gyrus. Paper speed, 7.5 mm. per sec.

A. Irregular activity characterized as "projection" activity, upon which bursts are superimposed.

B. After intercollicular section. Note the decrease in amount of irregular "projection" activity and improvement in bursts.

Projection activity in the cortex, when present, was correlated with activity of similar frequency and appearance in the ventrolateral thalamic nucleus. Figure 11 shows simultaneous records from thalamus and cortex from a preparation in which projection activity was a noteworthy feature. The amount of thalamic activity greatly exceeded that recorded from the same thalamic region in animals whose prevailing cortical activity was of the burst variety (fig. 5B).

Because the individual potentials of the projection activity did not repeat themselves at regular intervals, it was difficult to study interaction with other effects. Nevertheless, certain statements can be made. The repetitive response was produced ordinarily in preparations whose depth of anesthesia was only slightly greater than that necessary to depress the projection activity. The addition of more nembutal to such preparations abolished or reduced the repetitive activity. Furthermore, in occasional instances when the projection ac-

tivity did not disappear after deeper narcosis was induced, good repetitive sensory responses did not occur. The usual effect was the production of one or two potentials following the stimulus at intervals comparable to those of the repetitive response, but later events were obscured by the simultaneous presence of the projection activity.

3. *Repetitive sensory response and the recruiting response.* Stimulation of medial thalamic structures associated with the internal medullary lamina has been shown to produce long latency recruiting potentials which have many characteristics in common with the spontaneous 8–12 per sec. bursts (Dempsey and Morison, 1942a). The interaction of these potentials with the repetitive response was studied. Figure 12 shows that recruiting responses in no way interfered with the repetitive responses. Similarly, the inhibition of spontaneous activity by high frequency stimulation of recruiting areas did not affect the

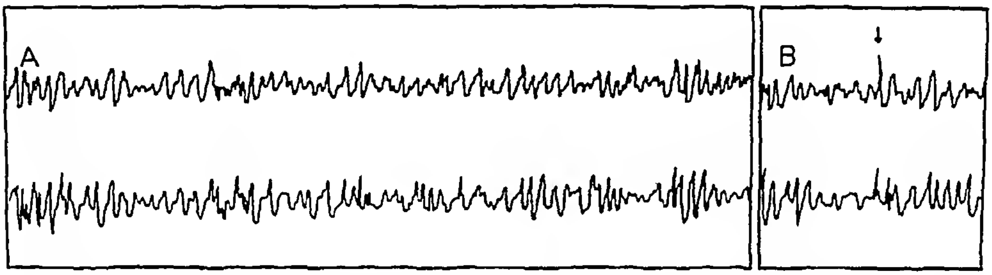

Fig. 11. Simultaneous presence of projection activity in cortex and thalamus. Upper record, *n. ventralis lateralis pars externa.* Lower record, radial cortical projection. Paper speed 7.5 mm. per sec.

A. Spontaneous activity. Note similarity of thalamic and cortical records. Compare with figure 1B for similar recording position when projection activity was absent.

B. Responses to single shock to radial nerve, applied at arrow.

repetitive response (fig. 12D), although such stimulation prevents the appearance of the spontaneous bursts (Dempsey and Morison 1942a).

4. *The augmented sensory response and spontaneous activity.* Application of a series of stimuli to the ventrolateral thalamus or radiations during a burst of spontaneous potentials led to augmentation of the cortical response regardless of the phase of spontaneous activity (fig. 9).

Less regularity has been encountered when augmentation was induced in the presence of projection activity. Application of a single shock anywhere in the sensory system ordinarily led to the appearance of only the "primary" potential in the cortical area while to evoke the augmented response repeated stimuli were required. Nevertheless, when single stimuli were applied to the lemniscus or capsule in preparations showing spontaneous projection activity, responses were produced, one component of which had a latency corresponding to element 4. The voltage of this effect varied depending upon the preceding activity. During and immediately after a spontaneous projection potential only the short latency phenomena (elements 1, 2 and 3) were seen. At longer intervals, responses at the latency of element 4 appeared. These effects sometimes were so large as to

overshadow the true primary response. In such preparations, stimulation of the nerve or sensory tract at a slow frequency (1 per 2 or 3 sec.) was followed by responses whose voltage varied depending upon the phase of projection activity at which the stimulus fell.

When two shocks were applied two or three hundred milliseconds apart, there was augmentation of the second response only when it fell at a time when no projection potential was present, although augmentation always occurred at these long intervals when the projection and repetitive effects were absent (cf. Morison and Dempsey, 1943).

Application of a series of shocks at slow frequencies to preparations in which projection activity was present produced no augmentation when the shock fell

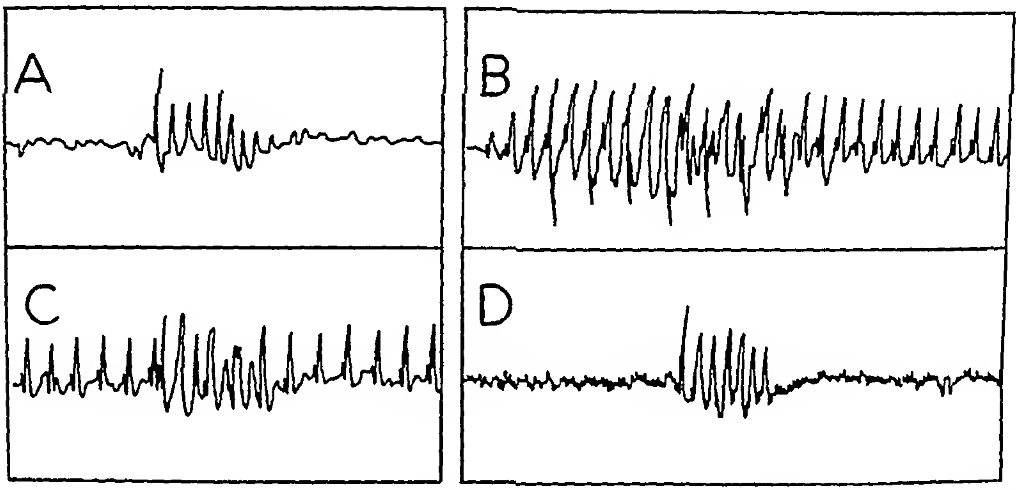

Fig. 12. Interaction of repetitive effect and recruiting potentials induced by stimulation of intralaminar thalamic regions (cf. Dempsey and Morison, 1942a). Radial cortical projection. Surface bipolar recording. Paper speed, 15 mm. per sec.

A. Immediate and repetitive potentials induced by a single shock to the radial nerve.

B. Recruiting effect of intralaminar thalamic stimulation. This effect rapidly equilibrates.

C. Repetitive response induced during equilibrated recruiting effect.

D. Repetitive response induced during 60 per sec. stimulation of the recruiting area. This high frequency stimulation abolishes burst activity (cf. Dempsey and Morison, 1942a).

upon a potential already existing. Stimulation at a rate of 7 per sec. usually led to block, an effect reminiscent of the superimposition of stimuli on the repetitive response. Faster frequencies, 8 to 60 per sec., invariably caused augmentation. Moreover, at these faster frequencies the projection activity was suppressed during the stimulation and occurred again upon its cessation.

Discussion. The possibility that certain components of the electrical activity of the brain may be separated, one from another, and studied as unitary phenomena has been emphasized previously (Morison and Dempsey, 1942). In the present paper, the possibility of separating spontaneous bursts from repetitive sensory responses has received especial attention because of their general similarity in form and sequence.

Several lines of evidence exist which reveal that the resemblances between spontaneous bursts and repetitive effects are only superficial. The conclusion that they represent states of activity in completely independent neuronal circuits may be summarized in the following manner. 1. The repetitive and burst potentials may be simultaneously evoked, an effect impossible if they utilized a common pathway (Sherrington, 1906; Dempsey and Morison, 1942b). 2. The cortical distribution of the two phenomena is different (p. 286). 3. The thalamic distribution of the two effects is separate (fig. 2). 4. Stimuli which modify burst activity do not affect the repetitive response (fig. 12). 5. The augmented response is blocked by repetitive potentials (fig. 7B), but not by burst potentials (fig. 9). 6. Eserine and acetylcholine markedly augment the repetitive responses while leaving the spontaneous bursts relatively unaffected (Chatfield and Dempsey, 1942; Morison and Dempsey, 1943). 7. The rhythm of the repetitive response is controlled by the delivery of the last shock of a series (Morison and Dempsey, 1943) while the rhythm of the spontaneous burst is not retimed by subsequent stimulation (Dempsey and Morison, 1942a). These considerations indicate, each with varying degrees of certainty, that spontaneous burst and repetitive responses reflect activity in totally different neuronal circuits. Taken together, their internal consistency enormously increases the probability of the conclusion.

There is also evidence that the neuron systems which mediate the repetitive and augmenting sensory responses can contribute an element to the so-called spontaneous activity of the cortex. Attention has been directed in the present paper to a type of activity that occurs in lightly anesthetized preparations to which the name "projection" activity has been applied. This type of activity is most dramatically revealed in the cortical areas upon which the great sensory systems project. It is ordinarily quite sensitive to anesthesia. Sections of the great afferent pathways abolish or greatly depress it (fig. 10). There are certain interactions between it and sensory responses which indicate neuronal linkages and common paths. Its presence in the cortex is correlated with activity of similar temporal characteristics in the ventrolateral thalamus (fig. 11). Conversely, absence in the cortex is accompanied by a reduction in the thalamic activity (fig. 5).

The characteristics of the projection activity listed above serve to distinguish it from the spontaneous 8 to 12 per sec. bursts which have been described previously (Derbyshire, Rempel, Forbes and Lambert, 1936; Dempsey and Morison, 1942a). The rhythmic bursts are best recorded from association cortical regions. They are quite resistant to anesthesia. Section of the afferent pathways never abolishes, but frequently enhances the rhythmic bursts (fig. 10). The various sensory responses can be superimposed with no suggestion of block upon the burst potentials. Their presence in the cortex is correlated with activity in medial rather than lateral thalamic structures (fig. 5; Morison and Finley, unpublished data). Lastly, stimuli which inhibit the spontaneous bursts either do not affect or actually enhance the projection activity (Morison and Finley, loc. cit.).

The data here presented permit the resolution of several difficulties which have obscured the mechanisms utilized in spontaneous activity and reconcile certain apparently contradictory statements in the literature. There is general agreement that section of the thalamocortical radiations abolishes or greatly depresses the spontaneous cortical waves (Bishop, 1936; Dusser de Barenne and McCulloch, 1938; Lewy and Gammon, 1940). Nevertheless, certain types of activity still persist after such cortical deafferentation (cf. Bishop, 1936; Bremer, 1938; Morison, Dempsey and Morison, 1941b). When sections of the afferent paths are made at lower levels, however, there is less agreement. Dusser de Barenne and McCulloch (1938) and Bremer (1938) have reported a persistence of spontaneous cortical activity after intercollicular section, while Lewy and Gammon (1940) report that with nembutal, but not with ether anesthesia, section of the afferent paths in the medulla or pons depresses and in the midbrain abolishes spontaneous potentials. Intermediate in position between these contradictory findings is the report by Dubner and Gerard (1939) that spontaneous potentials in the lateral geniculate bodies and optic cortex remain for one to two hours after deafferentation or maintenance in the dark, but can be reinitiated in the latter preparation by photic stimulation.

Similar confusion has attended attempts to interpret evoked potentials in thalamocortical circuits. Bishop (1936) and Bartley (1942) found that the visual cortex response to optic stimulation varied depending upon the phase of spontaneous activity present at the time. Moreover, on the basis of local strychninization of the somasthetic cortex and the ventrolateral thalamic nucleus, Dusser de Barenne and McCulloch (1938) suggested that a closed chain circuit existed between cortex and thalamus, and that spontaneous activity resulted from activity within this self-re-exciting system. Such a system could easily account for Bishop's and Bartley's observations that afferent impulses were blocked and facilitated periodically by spontaneous potentials. On the other hand, it is difficult to reconcile such a system with Marshall, Woolsey and Bard's report (1941) that ordinarily sensory potentials could be superimposed upon spontaneous waves, but that occasionally block occurred. Similarly, Dempsey and Morison (1942b) showed unequivocally that sensory potentials could occur with no reduction in size on any phase of the potentials of the spontaneous burst, and that these latter effects were intimately related to activity in medial rather than lateral thalamic areas.

If, as in this and preceding papers, "spontaneous" activity be regarded as a sum of several components rather than as a unitary phenomenon, there is a considerable resolution of the difficulties outlined above. For example, no contradiction exists between Bishop's statement that spontaneous potentials block an induced response and Dempsey and Morison's finding that evoked potentials may occur at any phase of spontaneous waves if it is assumed that in the former instance the spontaneous activity is of the projection type (p. 290) and in the latter the burst type. The observation by Bartley (1936) that optic stimulation induced a repetitive response which interacted with further stimuli supports the inference that projection activity is easily evoked in the visual system. Likewise, it appears probable that Lewy and Gammon were dealing with projection type

activity since it was influenced so markedly by section of the sensory tracts, while Bremer and Dusser de Barenne and McCulloch found that burst activity remained after intercollicular section. The interlinking of the cortex and ventrolateral thalamus observed by Dusser de Barenne and McCulloch after local strychninization, on the other hand, was clearly due to the projection circuit, since it was distributed to the lateral rather than medial thalamic areas and since it has been found that such cortical strychninization results in the firing of the strychnine spike whenever a sensory potential is induced in the strychninized area, while the potentials of the spontaneous burst do not trip the strychnine spike with such facility (Morison and Dempsey, unpublished data). Similarly, it appears probable that Dubner and Gerard were dealing with projection type activity in the lateral geniculate, since they observed a decline in magnitude on confinement to the dark and a re-establishment of activity on visual stimulation.

There are certain implications in the data presented above which bear upon the interpretation of cortical responses. Lorente de Nó (1939) has adduced evidence that an impulse traveling toward an "active" electrode induces in it a potential whose sign is positive when referred to a remote "indifferent" electrode. This interpretation has been followed by Curtis (1940) and Marshall, Woolsey and Bard (1941), although the latter authors observed that lemniscal stimulation produced in the cortex spikes of axonal dimensions followed by a positive wave. The evidence presented in figure 3 indicates that as opportunity for temporal dispersion is decreased, the surface positive component of the primary becomes resolved into two positive elements. The second of these elements declines faster with frequency than does the first. Likewise, the concomitant presence of an augmenting response causes a faster decline in element 2 than can be accounted for by fatigue of the afferent fiber (cf. fig. 2, Morison and Dempsey, 1943). These are phenomena which are difficult to explain on the assumption that both positive elements are the immediate electrical signs of corticopetal impulses. Rather, such discontinuities ordinarily attend transmission across a synapse, and it is therefore suggested that at least one cortical element must be activated during, and must contribute to, the surface positive wave which results from sensory stimulation.

SUMMARY

Stimulation of sensory elements at any level in the medial lemniscus-internal capsule relay system is followed by responses of three types in the sensory cortex and ventrolateral thalamic nucleus: 1, a "primary" response consisting of two positive and one negative deflections (figs. 1A and 3C and D) which follow rapid frequencies of stimulation; 2, an "augmenting sensory response" consisting of a positive and negative component which, although absent on the first stimulus, grows dramatically in size on successive stimulations (figs. 1B, 2A, 3A and 4); 3, a "repetitive sensory response" consisting of a train of potentials at a frequency of 8–12 per sec. which follows the primary response to a single stimulus and which fatigues quickly on repeated evocation (fig. 7A).

The primary and the augmenting responses have been separated from one another on the basis of latency (figs. 1 and 3), anatomical localization (fig. 2), selective abolition by lesions and differences in physiological behavior (p. 288).

Similarly, the separability of the primary and the repetitive responses has been demonstrated by the failure of one to block the other (fig. 6). On the other hand, the simultaneous presence of the repetitive response blocks the appearance of augmentation (figs. 7B and C).

The repetitive response, although superficially similar to the spontaneous burst potentials of the cortex, may be separated from them since the burst potentials occur in different areas in the thalamus (fig. 5) and since the presence of burst potentials does not prevent the evocation of repetitive responses (fig. 8). The validity of this finding is further indicated by the fact that the repetitive response is not affected by simultaneously induced recruiting potentials or by high frequency stimulation of intralaminar thalamic areas (fig. 12), although these procedures have been shown previously to interfere with the burst potentials. Similarly, the augmented response is not blocked by the burst potentials (fig. 9).

Evidence is presented that a type of spontaneous effect, the "projection activity," results from activity in the thalamic relay nucleus and its cortical projection. The projection activity differs from the spontaneous cortical bursts in regularity, susceptibility to anesthesia, thalamic localization (fig. 11) and dependence upon afferent impulses (fig. 10). Its interaction with the repetitive sensory response is complex (fig. 11B). It is abolished by rapid augmenting stimuli (p. 292), whereas augmenting responses are superimposable upon the burst potentials (fig. 9).

It is suggested that the recognition of different types of "spontaneous" activity facilitates study of the interaction of cortical potentials with induced effects. The bearing of these results upon various contradictory statements in the literature is discussed.

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MECHANISM OF THALAMOCORTICAL AUGMENTATION AND REPETITION

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In another paper two types of cortical activity elicited by stimulation of the sensory path have been described. Evidence was presented that these phenomena, the so-called augmented and repetitive sensory responses, share a single neuronal mechanism, but may, on the other hand, be segregated both from the simple "primary" sensory response and the generalized 8 to 12 per second "spontaneous" bursts (Dempsey and Morison, 1943). The present study was directed toward a more thorough investigation of the properties of the system responsible for the augmented and repeating effects.

METHODS. The methods employed were entirely similar to those employed in the preceding papers and need not be repeated.

RESULTS. *Site of repetition and augmentation.* Since repetition is not a feature of sensory responses recorded from the lemniscus (Dempsey and Morison, 1943), attention was directed to the thalamus and cortex. Adrian (1941) has demonstrated that the thalamus deprived of its cortex still exhibits repetitive responses. In the present investigation records taken from the thalamus before and after cutting of the internal capsule showed that the repetitive response of the thalamus, though still present after the procedure, was always depressed. In order to facilitate cutting of the capsule in this and other types of experiments, the ventricle was carefully unroofed before any observations were made, so that the cutting of the capsule could be carried out with a minimum of additional operative trauma. Since depression of the thalamic response might still be attributable to some nonspecific effects of the operation, however, the influence of the cortex on the thalamic response was tested in another way. Cortical repetition was enhanced by local application of acetylcholine (Chatfield and Dempsey, 1942). The thalamic repetitive response was enhanced *pari passu* (fig. 1).

The question still remained as to whether the cortex alone may exhibit repetitive or augmented sensory responses. In order to solve this question, stimulating electrodes were introduced into the capsule and the cortical responses recorded. Division of the capsular fibers caudal to the electrodes abolished both the repetitive and augmented (fig. 2) responses. Even though acetylcholine was applied to the cortex in the hope of compensating for the loss of an asynchronous thalamic bombardment (cf. its facilitating effect in intact animals above), the abolished repetitive response did not return.

The primary response induced by capsular stimulation invariably was smaller and its second and third component declined more rapidly with frequency before section of the thalamic radiations. As the augmenting response developed (fig. 2A), a decline in the primary response occurred. Figure 2B shows the larger

size and greater durability of the primary effect after section of the thalamic radiations.

At this point it may be recognized that capsular stimulation could presumably involve both dromic and antidromic activation of either corticopetal or corticofugal elements. Difficulties arising from antidromic stimulation have been so slight as to justify their complete neglect. Phenomena of short latency (0–1 msec.) and duration (1–2 msec.) did indeed occur both in the cortex and thalamus as a result of capsular stimulation (cf. Dempsey and Morison, 1943), but they had little effect upon the later augmented and repetitive responses. These early effects in the cortex were presumably due to direct activation of the thalamocortical fibers along with an undetermined amount of antidromic firing of corticopetal elements. At all events, it seems unlikely that a very large proportion of fibers carrying the augmented response to the cortex are activated directly by the electrodes in the capsule for the augmented response was typically very much larger than any early response to capsular stimulation. Indeed, from certain positions in the capsule virtually no cortical response other than the augmented one was detectable (cf. Dempsey and Morison, 1943).

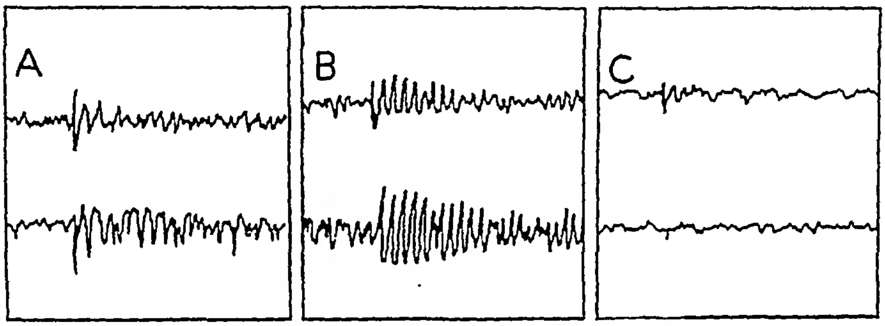

Fig. 1. Reinforcement of repetitive response by local application of prostigmine and acetylcholine to the cortex. Upper record, *n. ventralis lateralis pars externa;* lower record, radial cortical projection, surface bipolar recording. Paper speed, 10 mm. per second.

A. Immediate and repetitive effects in thalamus and cortex after single shock applied to the radial nerve.

B. After local application of prostigmine and acetylcholine to the cortex. The repetitive effect is enhanced in both cortex and thalamus.

C. After decortication by section of the internal capsular fibers. The thalamic repetitive effect, although still present, is greatly depressed.

Further evidence against attributing a significant rôle to direct activation of thalamocortical neurons is found in the observation that neither augmented nor repetitive responses could be produced in the cortex deprived of its thalamus. On the other hand, augmented and repetitive effects in the thalamus were easily elicited by capsular stimulation whether or not the cortex were present. The latency of the augmented response in the thalamus (2–3 msec.) was such that it preceded by an appropriate interval the corresponding cortical effect (3–5 msec.), an observation which lends cogency to the view that capsular stimulation exerts its effects on the cortex via the thalamus and not *vice versa* (fig. 3). Other

evidence which serves to establish the view even more satisfactorily may be found in the subsequent experiments.[1]

Rhythmic responses to high frequency stimulation. Relatively rapid frequencies applied either to the capsule or an afferent nerve resulted in a marked reduction of both the cortical and thalamic responses, a phenomenon due apparently to the

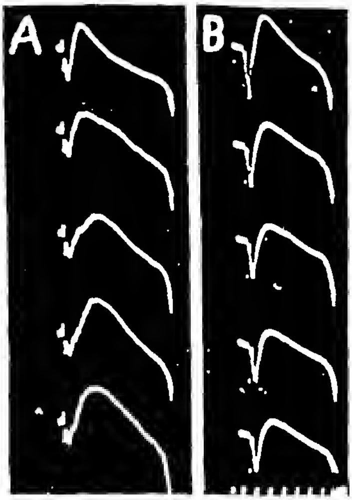

Fig. 2

Fig. 3

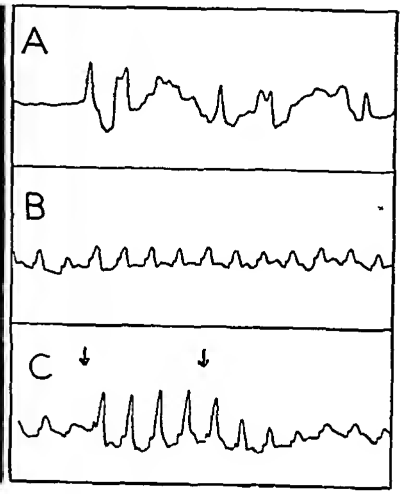

Fig. 4

Fig. 2. Abolition of augmenting response after removal of the thalamus.

A. Stimulus to the internal capsule. Primary and augmenting effects are present.

B. Same after section of the internal capsule posterior to the stimulating electrodes. The augmenting effect does not appear. The primary response is larger and does not decline so rapidly as in record A. Time signal, 250 cps.

Fig. 3. Simultaneous augmentation in thalamus and cortex during repetitive stimulation of the internal capsule. Left record, *n. ventralis lateralis pars externa.* Right record, radial cortical projection, surface to deep electrodes. Time signals, 100 cps. Note that the latency is shorter in the thalamic response.

Fig. 4. Interaction between capsular and afferent nerve stimulation. Radial cortical projection, surface bipolar electrodes. Paper speed, 40 mm. per second.

A. Effect of 120 per sec. stimulation of radial nerve. Note irregular repetitive discharges.

B. Equilibrated response to 12 per sec. stimulation of an augmentor point in the internal capsule.

C. One hundred twenty per sec. stimulation of radial nerve between arrows. Note increased responses to the capsular stimuli, and that this effect outlasts the period of nerve stimulation.

combined effects of fractionation and "fatigue." Superimposed upon the equilibrated series in many experiments a series of rhythmic waves of slow (8–12/sec.) frequency appeared both in the thalamus and cortex (figs. 4A and 5).

[1] The analysis here and throughout this study has assumed that the growth of the augmented response is produced by increasing the number of active elements. Owing to the exigencies of space, the somewhat elaborate discussion necessary to justify this procedure is omitted.

Again the effects of both capsular, lemniscal and afferent nerve stimulation were similar, except that the former were easier to obtain and more marked in extent. The rhythmic waves occurred in the thalamus deprived of its cortex, but not in the cortex without the thalamus.

Interaction of afferent and capsular stimulation. It has been shown elsewhere that the simple sensory primary response elicited from afferent nerve stimulation is "blocked" by prior activation of the appropriate portion of *n. ventralis lateralis pars externa* or of the internal capsule (Dempsey and Morison, 1942). Similar experiments involving the augmented response revealed much more complex interactions. Complete block has never been unequivocally encountered, but various degrees of mutual facilitation or occlusion have been the rule. Facilitation was strikingly illustrated in some experiments by setting up a cortical response by capsular stimulation at a frequency high enough to result in marked equilibration. An added stimulation of an afferent nerve at rates so high that

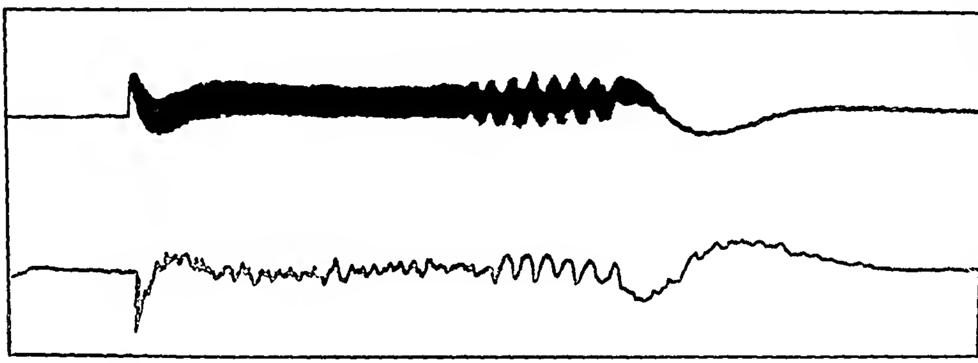

Fig. 5. Effect of high frequency stimulation of the internal capsule. Upper record, *n. ventralis lateralis pars externa.* Lower record, radial cortical projection, surface to deep recording. Paper speed, 15 mm. per sec. Stimulus frequency, 250 per sec. Note the development of oscillations in the records.

discrete responses to the individual volleys were undetectable yielded marked facilitation of the cortical response at the capsular frequency (fig. 4).

At slower frequencies the effects depended upon several factors including especially the time relations or phase angle between the two types of volleys. Less than lineal or greater than lineal addition of the effects were encountered in different conditions suggesting that the pool of thalamocortical neurons could be shared by the corticofugal and lemniscus fibers. Under conditions of marked equilibration or slight augmentation a large subliminal fringe was available to the other stimulus, and facilitation would result. With highly augmented or slightly equilibrated responses "occlusion" was revealed (i.e., less than lineal addition).

Time course of augmentation. One of the most interesting features of the augmentation effect was its time course. Figure 6 shows the effect of a second stimulus at varying intervals after the first. It is clear that augmentation was minimal shortly after the first stimulus and rose, first rapidly then more slowly,

to reach a maximum at 100 to 120 msec. immediately before the appearance of the first repetitive wave. All augmentation was abolished during the repetitive wave, but reappeared immediately afterwards to rise to another, frequently higher, maximum before the next repetitive wave and so on (fig. 7). The presence of the repetitive response made it difficult to determine the total duration of the augmentation produced by the first shock because the repetitive waves might have contributed to or subtracted from it.

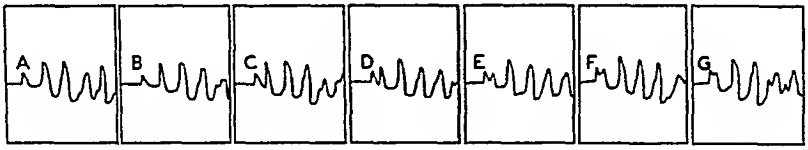

Fig. 6. Timing of the repetitive response by the second of two stimuli. Radial cortical projection, surface to deep electrodes. Paper speed, 20 mm. per second.

A. Immediate and repetitive effects of a single shock applied to the internal capsule.

B. Response to two shocks separated by an interval of 100 msec.

C. Sixty-seven msec.; D. 50 msec.; E. 33 msec.; F. 25 msec.; G. 20 msec.

The first repetitive potential occurs at a constant time after the second stimulus, regardless of its phase relation to the first.

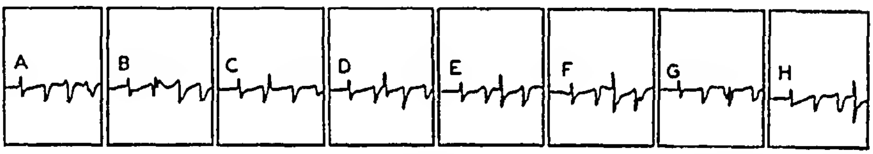

Fig. 7. Growth of augmentation during the interval between repetitive potentials. Radial cortical projection, surface bipolar electrodes. Paper speed, 20 mm. per second.

A. Immediate and repetitive responses to a single shock applied to the ventrolateral thalamic nucleus.

B. Block of augmentation when a second shock falls during the first repetitive potential.

C, D, E and F. Increase in augmentation as second shock falls later and later after the preceding repetitive potential.

G. Block of augmentation when second shock falls upon the second repetitive potential. Compare with B.

H. Augmentation when second shock falls after the second repetitive response. Compare with F.

Note that the repetitive effects are retimed by the second shock. The interval between the second shock and the next repetitive potential is constant, regardless of the phase at which the stimulus falls.

A somewhat similar waxing and waning of the responses of the optic cortex to the second of two stimuli has been discussed by Bartley (1936, fig. 3). In spite of the differences in cortical area, species, anesthesia and stimulating methods employed, the similarities between the two results suggest that a similar mechanism may be fundamental to both situations. It may be suggested that the second or slow response of Bartley and Bishop (1933) is analogous to the augmenting response identified here. As Bartley (1941) points out, the slow response is more intimately connected than is the earlier diphasic wave with spontaneous cortical activity, a point consistent with the augmenting effect of

Dempsey and Morison (1943, p. 291). On the other hand, it seems regularly to occur in the optic area in response to a single shock, whereas in the somatic sensory area it was more typically the result of repeated stimulation. This and other differences have led to rather different interpretations in the two cases, but it seems probable that as more information accumulates a single, general explanation may be possible.

In preparations without a repetitive response or in those in which it had been fatigued, augmentation as tested by two shocks usually recorded a maximum at 100 to 300 msec. and then slowly declined after the next second or two to normal, although in this condition the maximal possible effect was somewhat less than in the unfatigued preparation. Other facts which suggest that the early rising phase of augmentation is relatively uninfluenced by intercurrent discharges were brought out by the use of repetitive stimulation. At frequencies higher than that of the repetitive rhythm consecutive augmentation of the first several shocks was usually seen. Over a range of from 8 to 20 per second the curve relating height of the individual responses to time was relatively constant (cf. fig. 8) at least in its early phases. In other words, neither the slope of the rising phase nor the maximum height of augmentation was increased by increasing the number of stimuli per unit time At optimal frequencies the total duration was, however, longer than that produced by a single shock. As frequency increased, however, the curves of augmentation began to decline earlier, suggesting "fatigue" and the appearance of progressive fractionation. At frequencies above about 30 per second complexities appeared in the early part of the curve, suggesting that refractoriness and fractionation were the dominant factors.

Timing of the repetitive responses. Perhaps the most interesting feature of the repetitive response is the regularity of its timing. In the course of a single experiment the frequency of the beats remained surprisingly constant even when various procedures had greatly altered the height of each individual beat or the duration of the train.

The application of acetylcholine to the cortex greatly enhanced the responses both in individual height and in total duration not only in the cortex but also in the thalamus. The frequency of the discharge, however, was essentially unchanged. Conversely, repeated production of the repetitive response at intervals of about 3 to 5 sec. greatly reduced the duration of the discharge and the height of the constituent waves without influencing their frequency. Indeed as each individual discharge petered out, the waves declined in size but changes in the interval between them were insignificant. Furthermore, although the repetitive response was always greater when produced by stimulation of the internal capsule than as a result of afferent nerve activation, the frequency in both cases was similar.

The following summary statement appears to be justified. The mechanism responsible for the repetitive response is timed by some subsidiary mechanism relatively inaccessible to the excitatory state upon which duration of after discharge or the height of the individual waves depends.

Another extraordinary feature of the mechanism was revealed when two single shocks were given at varying intervals. The experiment illustrated in figure 6 in which the stimulating electrodes were in *n. ventralis lateralis pars externa* was typical; similar results have been obtained by the stimulation of afferent nerve or capsule. The first stimulus set up the repetitive train. No matter when the second shock was given and irrespective of how small the response elicited, the interval between it and the next succeeding repetitive wave was constant, i.e., the timing of the remainder of the train was set by the second stimulus. The total duration of the train as measured from the first stimulus, however, was but slightly if at all increased by the addition of the second shock. In other words, a second shock could alter the timing of the series but did not contribute significantly to the duration of the excitatory state.

As the stimulus interval was decreased below 20 msec. the second shock became less efficient at taking over the timing, and irregularities appeared in the repetitive waves suggesting that the pool of thalamic neurons was split between the two stimuli, but beyond this limit the rule outlined above was strictly followed. Similar results were obtained if three or even four shocks were used; the last one always set the remainder of the rhythm. In the multiple shock experiment the train was usually somewhat shortened or even completely abolished.

An exception to the statement made above that a second shock did not contribute to the excitatory state responsible for repetition should be mentioned. A weak stimulus to the capsule would sometimes be followed by a repetitive response smaller and shorter than "maximal." Such responses were increased by the addition of a second shock at some reasonable later interval. The gradations between submaximal and maximal responses were not smooth, but tended to occur in steps. This fact which suggests that the elements controlling repetition are relatively few in number was reinforced by other observations. Many of the repetitive discharges tended to show constant irregularities in the constituent waves which suggested that they were made up of two or more units firing slightly out of phase. As the discharge disappeared, the height of the original waves tended to decline by steps and not by a continuous decline (cf. Dempsey and Morison, 1943, fig. 7A). The splitting of the pool between two stimuli arriving a short time apart mentioned in the preceding paragraph also reinforces the suggestion that the repetitive mechanism is made up of relatively few "all-or-none" units.

Discussion. The experiments described in this and accompanying paper suggest that a system in addition to the simple lemniscus to internal capsule relay is present in the ventrolateral thalamus. It would appear that extensive afferents to this thalamic system are derived from the cerebral cortex and the medial lemniscus. Moreover, these afferents apparently do not end upon the ultimate thalamocortical neurons, but exert their effects through interneurons. Finally, this system of thalamic afferents, interneurons and efferent cells has certain properties, typified by a slowly rising excitatory state and by prolonged rhythmic activity, which are pertinent to a discussion of the possible anatomical and physiological organizations underlying the various phenomena.

In order to summarize the data and point up its implications, it may be helpful to consider a diagram (fig. 9). Clearly in such a complex system data can only be cited very tentatively as favoring one or another of the contested views in regard to the intimate nature of synaptic transmission, facilitation, c. e. s., c. i. s., or after discharge.

As Lorente de Nó (1939) has shown, many of the properties attributed by others to c. e. s. and "detonator" activity may be reduced to temporal and

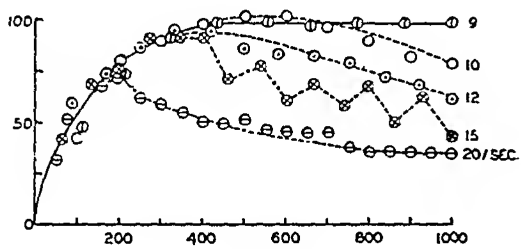

Fig. 8

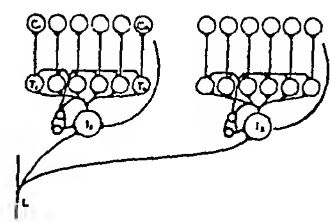

Fig. 9

Fig. 8. Time course of cortical augmentation at various frequencies. Ordinates, height of successive responses at indicated frequencies, expressed as per cent of maximal augmented response. Abscissae, milliseconds after first stimulus. The rate of increase in augmentation is independent of the frequency of stimulation, although the decline in height during equilibration is greater and occurs earlier as the frequency is increased.

Fig. 9. Schematic representation of the system responsible for augmented and repetitive effects.

A homologous group of interneurons, I_1, $I_2 \ldots I_n$, interposed between capsular and lemniscal afferents and thalamocortical efferents, is postulated to fire on any afferent stimulus. These interneurons distribute to thalamocortical efferents $T_1 \ldots T_n$, each element of which requires successively greater degrees of facilitation for its discharge. Since the degree of augmentation is a function of time rather than frequency of stimulation, the excitatory state is represented as a self-re-exciting chain which is started by the discharge of I and which facilitates transmission at T_n. Any afferent stimulus therefore fires I and the portion of T_n previously facilitated by the closed chain.

Since the system fires repetitively after a single stimulus, the closed chain is regarded as symbolizing the summatable excitatory state involved in rhythmic firing of I (see text). The discharge of I, so induced, fires all of T_n because its excitatory state induced by previous activity of the closed chain is maximal at the period of the repetitive effect. This group discharge of T_n abolishes its excitatory state thus temporarily abolishing augmentation to an interpolated shock. Furthermore, the entire system is retimed by the abolition of facilitation at I following its discharge (see text).

The origin of return fibers from the cortical cells C_n is unspecified, since it is probably several neurons removed from the element on which the thalamocortical fibers terminate.

spatial patterns of activity in interneurons. Although the phenomena presented here can apparently be explained in these terms, such a diagram becomes very complex, and, as Lorente de Nó has pointed out, loses therefore much of its didactic value. A rigidly complete analysis in those terms has therefore not been attempted.

The key to the present system lies in the fact that the timing mechanism of the rhythm of the repetitive response bears a highly specialized relationship to the afferent fibers (either capsular or lemniscal) on the one hand, and to the

efferent or thalamocortical fibers on the other. The principal point of interest is that the entire system may be retimed by stimuli which may activate relatively few of the thalamocortical fibers (fig. 6). It seems desirable, therefore, to postulate at least one group of homologous interneurons I_n between afferents and efferents, which may be thought of as completely accessible to successive volleys arriving at intervals so short as to be within the unresponsive period of the thalamocortical pool T_n. This group may be taken as symbolic of a much more complex interneuronal system, primarily concerned with control of the rhythm.

The fact that the rhythmic waves of the repetitive response could be resolved in favorable instances into a relatively small group of units which tended to appear or disappear in a relatively "all-or-none" manner has suggested the representation of the interneuronal system as composed of a few large cells receiving many corticothalamic and lemniscus afferents and relaying to a large number of thalamocortical efferents. The smoothly graded nature of the augmented response may then be attributed largely to recruitment in the large pool of the latter type, although facilitation at both I_n and T_n would of course be important with weak capsular stimuli or in the case of activation of afferent nerves (cf. p. 303). For conveniently analyzing recruitment at T_n, stimuli maximal for I_n are therefore desirable, and the data detailed in figures 6 and 8 have been obtained with intensities such that temporal effects at I_n may be neglected. Started by a single volley, augmentation slowly rose for 100 to 300 msec. and then slowly disappeared. The rising phase and total amount of augmentation have further been shown to be relatively uninfluenced by additional volleys, although the duration of the effect may be prolonged by repetitive stimulation (fig. 8).

The excitatory state responsible for augmentation has, therefore, been represented as a self-re-exciting chain which is started by a single shock and continues to run for 2 to 3 seconds with a rate uninfluenced by successive volleys within that interval. Activity in this system may be thought of as progressively lowering the threshold of T_n so that more and more of them become available to the second volley arriving over I_n. A strict interpretation of the concept of Lorente de Nó would apparently rule out such an accumulation and a more complicated system of delays would be necessary. On the other hand, the shape of the time curve and the lack of evidence for actual firing of T_n by the facilitating process alone suggest the c. c. s. postulated by Eccles (1936).

It is now possible to consider the characteristics of pool I_n which is made responsible for the rhythmic properties of the system. In addition to relaying impulses to pool T_n as outlined above, pool I_n is postulated as firing rhythmically under proper conditions. At present the whole problem of rhythmicity can only be discussed in vague terms, but the phenomena considered here are most readily understood if one assumes that the rhythmic firing of I_n is due to the interaction of an extrinsic excitatory effect represented by a collateral from the reverberating side chain already postulated to account for facilitation at T_n, and a non-summatable recurring refractoriness or subnormality of constant

duration. The arrangements necessary for rhythmic activity are clearly more specialized and labile, however, than are those of the simple relay activity. In general more impulses must reach I_n to elicit the repetitive response and it is distinctly more affected by fatigue. No matter what the mechanism of rhythmicity, the accurate retiming of the system by a second shock raises an interesting point (figs. 6 and 7, p. 301). All the interneurons controlling T_n are affected by the interpolated shock whether or not T_n are fired thereby since no wave appears at the usual interval after the first stimulus. On the other hand, the entire pool fires repetitively at the usual interval as measured from the interpolated stimulus. The constancy of the interval between interpolated discharge and first repetitive wave differentiates the phenomenon from the retiming of rhythmic motor neuron discharge described by Eccles and Hoff (1931). In their instance the interval succeeding the interpolated discharge varied inversely with the length of the curtailed cycle, a situation which appears easily explicable on the basis of summation of subnormality or an interaction of N and P waves (Eccles, 1936). The present case, though superficially simpler, is actually more difficult to explain, and necessitates the non-summatability of the subnormal state postulated above, which just cancels the accumulated facilitation.

The fact that repetitive responses are more difficult to elicit by afferent nerve or lemniscal stimulation than from the capsule has led to the supposition that the lemniscus fibers relaying impulses from a particular afferent nerve are sparsely represented on the I_n cells. On the other hand, when properly facilitated (i.e., by application of acetylcholine and prostigmine to the cortex) a very large part of I_n becomes available. The inference is that any portion of the lemniscus though poorly represented on any particular part of I_n is distributed widely through the pool. Cross linkages between the interneurons I_n could also contribute to the spread of effects and are rendered quite probable by the synchronization of the waves in the repetitive response and the presumably similar rhythmic waves produced by high frequency stimulation. A mechanism for avalanche conduction and the more elaborate interpretations discussed by Marshall and Talbot (1941) is provided by either sort of connection. Clearly also an opportunity for the various forms of interaction between lemniscal and capsular stimulation outlined on page 300 is offered by the overlap of these two types of afferents on the I_n cells.

It is interesting to note that although interactions between capsular and afferent nerve stimulation reveal various states of facilitation as well as occlusion of the augmented response, the true primary (elements 1, 2, 3 of Dempsey and Morison, 1943) exhibits occlusion only (Dempsey and Morison, 1942). This and other evidence such as the independence of the primary response from spontaneous activity in any recognized corticothalamic circuits suggest that corticothalamic fibers are poorly represented on the neurons responsible for the relaying of the simple primary response.

Presumably the repetitive waves recorded in response to high frequency stimulation of either capsule or afferent nerve (p. 299) is due to a piling up of excitation at these interneurons. At these high rates responses of the I_n cells to the indi-

vidual volleys seem to be prevented probably by the postulated subnormality which allows only the much slower rhythmic waves to appear in response to an accumulated excitatory state.

In conclusion it may be worth while to turn for a moment to the problem of corticothalamic interaction. From the evidence presented (p. 297) it is clear that the principal mechanism underlying augmentation and repetitiveness lies in the thalamus. The fact that the rhythm of the repetitive response of the thalamus is unaltered by decortication (p. 298) emphasizes that the timing is localized in the thalamus and is not a property of the circuit as a whole. On the other hand, the cortex contributes importantly to the intensity of the response and to its duration, presumably by facilitation fed back to I_n and perhaps to T_n. Thus, though the circuit may not be strictly "reverberating," it may properly be referred to as mutually reinforcing. The relation of this system to an element of the spontaneous electrocorticogram and the fundamental experiments of Dusser de Barenne and McCulloch is discussed elsewhere (Dempsey and Morison, 1943).

CONCLUSIONS

Investigation of the mechanism underlying the production of repetitive and augmenting sensory responses has revealed the following:

Decortication reduced but did not abolish the repetitive response recorded from the thalamus. Enhancement of the cortical response by local application of drugs increased the thalamic effect (fig. 1). Removal of the thalamus abolished the repetitive and augmented responses evoked by capsular stimulation. These and other experiments (cf. figs. 2, 3, 4, 5 and p. 300) show that extensive thalamic afferents are derived from the internal capsule as well as the medial lemniscus. It is concluded that in this system there is a mutual reinforcement of cortical and thalamic effects, but that the thalamus alone is necessary for the occurrence of repetitiveness and augmentation.

Augmentation induced by a conditioning shock, and revealed by a second test shock, extends over several hundred milliseconds (p. 301; figs. 6, 7 and 8). Furthermore, once the process is started, it proceeds at a rate which is not influenced by subsequent stimulation (fig. 8).

Repetitive sensory potentials induced by the conditioning shock destroy augmentation. Following the repetitive effect, the process of augmentation again builds up to a maximum just before the next repetitive response (fig. 7).

The timing of the repetitive rhythm bears a highly specialized relationship to the afferent stimuli. The rhythm is timed by the last stimulus, whether it falls during the repetitive train (fig. 7) or ahead of the first repetitive potential (fig. 6). Likewise, the rhythm is set by the last shock both when the number of thalamocortical elements fired is small (fig. 6 E, F and G) and large (fig. 6 B, C and D).

The data presented above imply certain properties of the thalamocortical system which are summarized (p. 303) and discussed with reference to a circuit diagram (fig. 9) whose elements are to be regarded as symbolizing such physiological phenomena as the excitatory state, detonator action and repetitiveness.

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CORRELATION OF VASCULAR CHANGES WITH CHANGES IN MOTOR ACTIVITY AND SECRETION IN THE STOMACH OF MAN

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The subject of our investigation was a man with a permanent gastric fistula 3.5 cm. in diameter, larger than that of Alexis St. Martin (1) or any other which has been studied in the past. His defect was the result of an operation done in 1895 and made necessary by a benign stricture of the esophagus. Despite the large communication to the outside world the appearance of the gastric mucosa both by direct inspection and gastroscopically was found to be normal (2). Secretory and motor functions of the stomach were average (2). Protruding through the defect and reflected on the abdominal wall around it is a collar of gastric mucosa. The width of this collar is made to vary with change in intra-abdominal pressure.

METHOD. Our subject came to the laboratory in the morning in a fasting state, and reclined for one-half hour on a cot before observations were begun. An effort was made to keep the subject's surroundings as neutral as possible and to keep him lightly diverted.

Circulatory changes. Changes in vascularity were readily recognized by variations in the color of the gastric mucous membrane. It was seen to undergo a very wide range of color changes under various circumstances, from a pale yellowish pink to a deep cardinal red. Changes of the same order and in the same direction occurred in the part of the mucosa within the cavity of the stomach and in that which lay exposed on the abdominal wall. Since it was simpler to obtain ideal lighting conditions on the outside, and since there a color scale could be brought up close to the mucosa for comparison, the recorded vascular changes are those observed in the collar of exposed mucous membrane under a cool, "soft white" fluorescent light suitably placed to provide constant lighting conditions. The standard colors used for comparison were quantitated by the method of Munsell. For convenience, however, the colors were given values from 10 to 100. The lower numbers corresponded to pale colors and the higher ones to deeper shades of red. It has been shown by a modification of the thermal gradient technic, described elsewhere (3) that these color changes do in reality reflect changes in blood flow.

It is important to note that accelerated blood flow in the mucosa was not merely associated with blushing of its surface. The membrane itself became wet, swollen, and turgid, and the rugae were slightly fuller and smoother. These evidences of vascular engorgement were especially obvious in the collar

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of mucosa exposed on the abdominal wall. During marked hyperemia, it often doubled in thickness from 5 mm. to 10 mm. and its radial folds decreased in number from 12 or 13 to 5 or 7. The tissue itself under such circumstances felt succulent and boggy.

Recording of gastric contractions. Records of contractions were obtained by the familiar technic of recording pressure changes in a balloon introduced into the stomach. It must be emphasized that this type of recording device measures only those contractions which alter the intragastric pressure. It has been suggested that certain peristaltic waves may course along the stomach without causing a change in intragastric pressure (4).

Collection of gastric juice. Continuous aspiration of the stomach contents was carried out through a small rubber tube accompanying the balloon. Collections made thus, when the walls of the stomach were held apart by the presence of the balloon, effected satisfactory emptying of the stomach. In the absence of the inflated balloon, however, the gastric juice collected in isolated folds of the collapsed stomach and was thus not thoroughly accessible to aspiration through a tube.

The completeness of the collection when the balloon was in place was confirmed by removing the balloon after collection of secretion through the tube, and rolling the patient over on his left side approximately 120°. No additional fluid poured out.

Analysis of gastric juice. The gross appearance of the specimens was noted. The degree, if any, of discoloration with bile was recorded. The relative concentration of mucus was estimated by noting the comparative viscosity of the fluid and the presence of shreds. Peptic activity was determined by the method of Mett (5). The concentration of acid was estimated by titration of the specimens against 0.1 N sodium hydroxide with Toepfer's solution and phenolphthalein as indicators of "free" and "total" acidity, respectively.

Estimation of parietal cell output. No method was available for the estimation of the output of hydrochloric acid by the parietal cells. Since acid is manufactured only by the parietal cells, however, it seemed clear that total hydrochloric acid was some function of volume of juice secreted within a specified time and the concentration of acid. Based on the relationship between hydrochloric acid and neutral chlorides in gastric juice established by Hollander (6), Gray (7) and others (8) (9), a formula was devised for the calculation of the approximate output of the parietal cells in terms of cubic centimeters of 0.166 N hydrochloric acid.

Reproduced in figure 1 is a modification of the curve established by Hollander for the relationship between hydrochloric acid and neutral chlorides in the gastric juice. Reference to the figure will show that the lower end of the line which relates the 2 constituents points near 166 millimoles of hydrochloric acid (total titratable acid of 166). A specimen of gastric juice containing this concentration of acid would consist of pure parietal secretion. At the other end of the curve, the constituents of the alkaline component are at a maximum, and there is no parietal secretion present. At a point half way along the ordi-

nate and abscissa, we are dealing with a solution containing a 50 per cent concentration of each of the constituents. Perpendiculars dropped from each of these points intersect at the midpoint of the curve. Therefore, at suitable intervals along the curve, perpendiculars may be dropped onto the abscissa to denote what per cent of solution of certain titratable "total acid" is actually made up of parietal cell secretion. Figures for total acid, of course, correspond numerically to millimoles of hydrochloric acid. Multiplying the percentage figure by the volume of gastric juice secreted within a given time yields the approximate quantity of parietal cell secretion elaborated during that period.

Sources of error in calculation for parietal cell output. The value thus obtained would, of course, not be entirely accurate since the curve illustrated was estab-

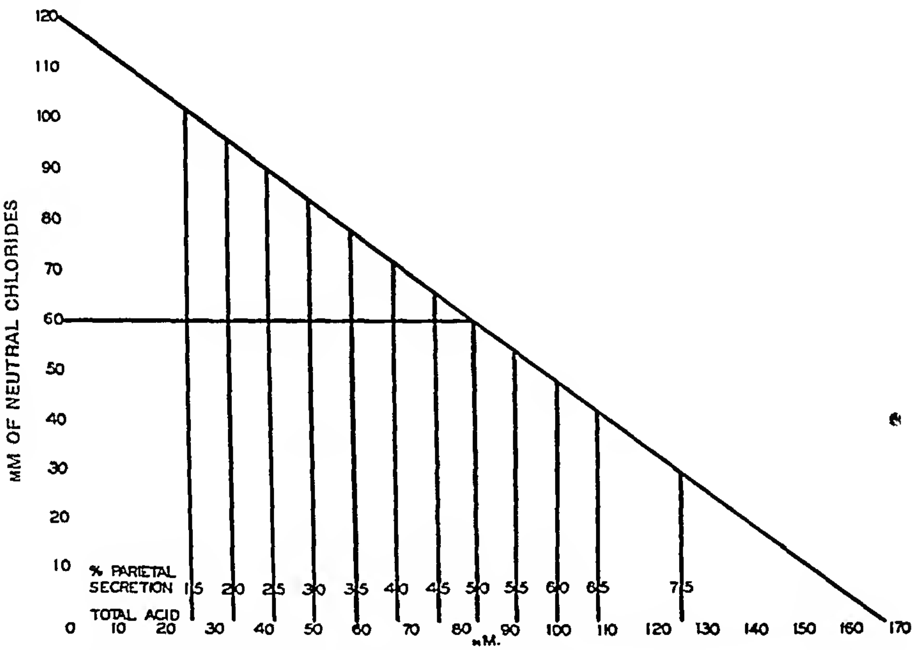

Fig. 1. Curve illustrating the relation between neutral chlorides and hydrochloric acid in the gastric secretion. (Modified from Hollander.)

lished on the basis of a statistical relationship, and individual specimens must be expected to vary slightly from the mean. Furthermore, the calculations were based on analyses of pure gastric juice in a blind stomach pouch. Any admixture of the juice with saliva or bile or any loss of juice through the pylorus would introduce a source of error. In this particular case, of course, the gastric juice could not be contaminated by saliva since the esophagus was completely occluded. Contamination of the juice with duodenal contents was easily recognized by the bile tint and such specimens were not included in calculations of parietal cell activity. Loss of secretion by emptying through the pylorus, however, was a persistent source of error. It always influenced the calculations in the same direction, however, and since emptying occurred only during periods of active motility (2), in the absence of vigorous gastric contractions the error became negligible.

Correlation of functions. 1. *Basal state.* Carlson (10) has shown that during the resting "basal" state the stomach undergoes periodic phases of intense contractile activity alternating with phases of relative quiescence. This same periodicity of function we have noted in the activity of the acid secreting cells and in the vasomotor activity in the stomach.

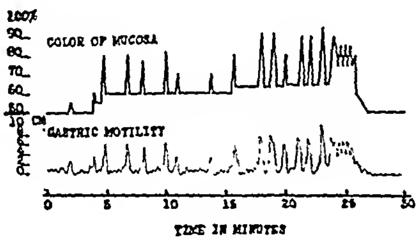

Fig. 2. Typical pattern of a phase of accelerated gastric motility with characteristic ending in a period of incomplete tetanus. Note changes in color of the mucosa associated with vigorous contractions.

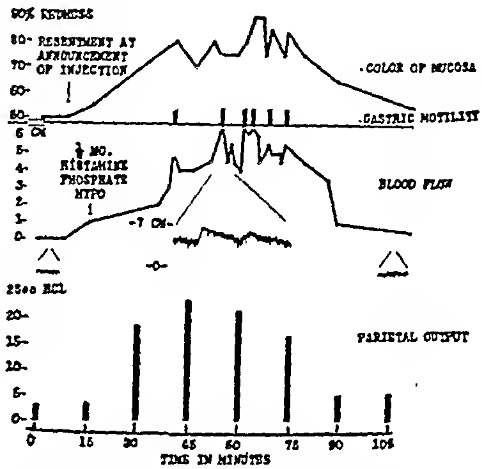

Fig. 3. Correlation of gastric functions including recorded blood flow following histamine injections.

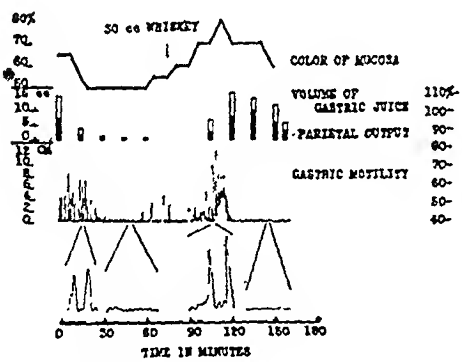

Fig. 4. Effect of alcohol on gastric function. Whiskey was administered following a period of spontaneous accelerated gastric function. Normally, a second such period would not follow for at least 2 hours.

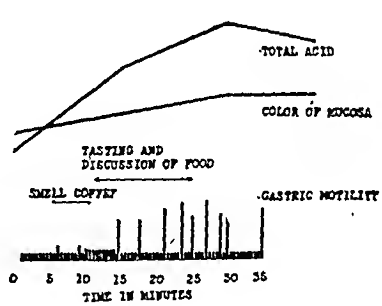

Fig. 5. Gastric function associated with stimulation of appetite.

Figs. 2–5

In the above figures the designation "Parietal output" indicates the volume of HCl at concentration 0.166 N.

The "basal" waves of low amplitude were not found to be accompanied by any detectable change in the color of the mucous membrane of the stomach. The more vigorous contractions, however, were associated with a simultaneous transitory blushing of the mucosa proportional in degree to the force of the contraction. Figure 2 shows the correlation of color changes in the mucosa with vigorous contractions of the stomach wall.

Like blood flow the rate of acid production in the stomach was accelerated

during phases of vigorous contractions and relatively slow during quiescent phases.

Comment. The occurrence of these spontaneous periodic phases of accelerated gastric function was carefully taken into account when vascular changes and changes in secretion and motility were attributed to the influence of stimuli applied experimentally. Usually such stimuli were applied shortly after the termination of one of these phases, when another would not be expected for an hour and a half. In addition, the experiments were repeated sufficiently often to establish the validity of the inferences.

Agents well known to cause an acceleration of acid secretion were tested for their effects on motor activity and vascularity in the stomach.

Effect of histamine on blood flow, acid secretion and motor activity. During a half-hour control period the gastric juice was collected and the color of the mucosa was observed. In addition a thermal gradientometer described elsewhere (3) was in place inside the stomach to measure changes in blood flow. Thus it was possible to correlate blood flow with color changes.

After the establishment of a base line, 0.0005 gram of histamine phosphate was injected hypodermically.

The results are charted in figure 3. Within 5 to 10 minutes of the injection the red color of the mucosa became deeper and there was a parallel increase in recorded blood flow. At the same time acid output increased in terms of parietal cell secretion and total titratable acid. It can readily be seen in the figure that the parietal cell output parallels the vascular changes.

Within 25 minutes after histamine injection strong contractions of the stomach wall occurred. With each of these there was a transitory blushing of the mucosa and an increase in recorded blood flow.

The maximum effect from histamine in terms of secretion, blood flow and motility occurred in about 45 minutes. After 90 minutes the effects had nearly subsided and the values had returned to normal by 2 hours.

Comment. These data illustrate: 1. That vascular changes observed as variations in color of the exposed gastric mucous membrane actually reflect changes in blood flow. 2. That histamine stimulated an increase in blood flow and motility as well as in acid production. 3. The importance of expressing acid output in terms of parietal cell secretion, since the "total acid" concentration failed to fall to control levels following a phase of accelerated secretion when acid output had subsided.

Effects of alcohol. After establishing a base line of vascularity, secretion and motor activity, 30 cc. of 90 proof whiskey (45 per cent alcohol by volume) was introduced into the stoma. As in the case of histamine an acceleration of all three functions occurred. The maximum effect was observed in 45 minutes, and in 90 minutes the values had returned to normal. A typical experiment is illustrated graphically in figure 4.

Comment. Other workers (10, 11) have shown that alcohol need not be introduced directly into the stomach in order to stimulate the parietal cells to accelerated activity. Given intravenously or rectally the drug causes a

sharply increased output of acid. Our observations indicate that even when ingested, the effects are not the result of local stimulation. Hyperemia of the mucosa and accelerated secretion did not occur until the drug had had time to become absorbed.

Effects of beef juice. Similar observations were recorded when a beef bouillon cube was introduced into the stoma. Within 15 minutes there occurred a flushing of the mucosa with enhanced secretory and motor activity which lasted more than an hour.

Preparation for eating. Another circumstance known to be associated with accelerated acid output is the feeling of good appetite which accompanies the sight or mere thought of delectable food. This was found to be also associated with hyperemia and hypermotility as illustrated in figure 5. The vascular engorgement, accelerated acid production and increased motor activity occurred promptly following the mention of appetizing food. Actual presentation and tasting of the food added little to this effect.

Hunger. Although, occasionally, hunger pains were reported by the subject at times in the absence of stomach contractions (2), as a rule they were found to be accompanied by vigorous contractions and a relatively red, actively secreting mucosa.

Correlation of blood flow, acid production and motor activity. *General statement.* While profound changes in blood flow, acid production and motor activity have been observed accompanying various emotional states and other circumstances, never in this subject were changes in vascularity and acid secretion dissociated. An increase in one was found under all circumstances to be associated with an increase in the other. Conversely, low acid output and pallor of the mucosa have been regularly associated. Vigorous motility has never been observed when the color of the mucosa was 50 or below. In the presence of red mucosa, vigorous contractions might or might not occur. No correlation was observed between the degree of hyperemia and the height or frequency of contractions, but accompanying each one was noted a transitory increase in redness of the mucosa, which subsided with the relaxation of the stomach wall.

Discussion. Doubtless, enhanced secretory and motor activity in the stomach requires added work by the cells of the stomach wall. Davenport and Fisher (13) have shown that in secreting acid, work is done by the cells of the gastric mucosa to the extent of 800 calories per liter of secretion. Others (14, 15, 16, 17) have found in animals and humans low values for gastric acidity during states of anoxemia induced by high altitude, low oxygen tension chambers severe hemorrhage, and even polycythemia. Correction of the anoxic state resulted in a return of acidity values to normal.

All of this suggests that hyperfunction of the stomach would be accompanied by hyperemia, and indeed it is a general biological experience that heightened cellular metabolism requires increased blood flow.

A few investigators (18, 19) have recorded combined measurements of blood flow and gastric secretion in animals. Lim, Necheles and Ni (18) studied gastric blood flow in the excised dog's stomach, perfusing it with another dog's

blood. They demonstrated acid secretion following histamine injections. The blood flow, as measured by cannulating the veins, failed to parallel the rate of secretion.

Dodds and his co-workers (19) made similar studies on dogs after surgical intervention. Instead of resecting the stomach, they left it in place and resected the entire small and large intestine. Following this, appropriate vessels to the stomach were cannulated, and the blood flow was measured by collecting the venous blood in a graduated container for a certain length of time. The blood was then transfused back into the animal. Their findings differed widely from those of Lim et al. An increase in blood flow was noted with an increase of volume of gastric juice secreted in response to histamine injection. They were able to inhibit the histamine effect with regard to volume of gastric secretion and blood flow by giving injections of pitressin or barium chloride, or by causing a vasoconstriction in the stomach by irrigating it with cold water. The titratable acidity, however, remained high.

In the interpretation of all of these animal experiments, one must reckon with the trauma of surgical operation, the effects of anesthesia, and the pathological changes secondary to the operative procedure.

A few observations have been made on circulatory changes in the gastro-intestinal tract by direct inspection of the serosal and mucosal coats. Kuntz and Hazelwood (20) found that a reflex vasoconstriction in the serosal coat of the intestine of the cat was induced by packing ice on the abdominal wall. Warming the abdomen was found to cause a vasodilatation in the serosa.

Beaumont (1) recognized in Alexis St. Martin that the stomach lining was redder at some times than at others. "On the application of aliment," he wrote, "the action of the vessels is increased, the color brightened. . . ." Anger and fear, he noted, produced a "morbid" appearance of the mucosa, although it is not entirely clear from his notes whether under those conditions the stomach was redder or paler. Carlson (10) observed in his Mr. V. a deepening of the red color of the mucosa with each strong contraction of the stomach wall. Schindler (21) has described the appearance of the stomach mucosa of patients with peptic ulcers as appearing redder than "normal" through the gastroscope. This instrument has not been left in place long enough to follow changes in color for more than a few minutes at a time.

Drury, Florey and Florey (22) studied vasomotor changes in a patch of exteriorized colon in dogs. They found with alarm or fright a uniform blanching of the mucosa, due presumably to constriction in the large branches of the mesenteric arteries. Barcroft and Florey (23) using similar preparations found pallor marked during brief spurts of running, but less marked during prolonged exercise. They also found pallor in association with "anxiety" or "excitement."

Our studies support these findings—that profound vascular changes occur in the gastric mucosa and, in addition, that hyperemia occurs in association with accelerated acid secretion and motor activity, and pallor with a decrease in these functions.

SUMMARY. Simultaneous observations of motor activity, vascularity, and

secretion in the stomach have been recorded. During the resting state, the stomach was found to be relatively pale and contracting with rhythmic waves of low amplitude. Acid was secreted continuously in small amounts. From time to time there occurred spontaneous phases of hyperemia, hypermotility and hypersecretion.

The effects of histamine and other stimuli on the various gastric functions were recorded. A direct association between vascularity (blood flow) and acid production was found to obtain under all circumstances of accelerated or depressed activity. Vigorous contractions, while they did not invariably accompany redness of the mucosa, never occurred in the presence of pallor.

CONCLUSIONS

1. Studies have been pursued on a subject with a large gastric fistula whose peculiar defect provided the opportunity to collect at intervals the total volume of secretion in the stomach.

2. The rate of acid production in the stomach was estimated under varying conditions with reference to volume of secretion and concentration of acid. The results were expressed in terms of parietal cell output.

3. Increased parietal cell output was always associated with hyperemia of the gastric mucosa. Hyperaemia also accompanied increased motor activity.

4. On the basis of data gathered from these studies, certain generalizations are possible with regard to the interpretation of the findings on routine gastric analyses performed on patients.

a. When an unusually large volume of gastric juice of high titratable acidity accumulates in an unobstructed stomach during a specified interval, it is safe to assume that the mucous membrane is relatively red and that no vigorous contractions are taking place in the stomach.

b. When a small volume of gastric juice of very high titratable acidity is obtained under similar circumstances, it is likely that the mucous membrane is relatively engorged with blood and that, in addition, especially vigorous contractions are taking place.

c. Low acid values in the presence of small volumes of gastric juice strongly suggest that the gastric mucosa is relatively pale and that no vigorous contractions are taking place.

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PHOSPHORUS DEPOSITION IN THE EGG AS MEASURED WITH RADIOACTIVE PHOSPHORUS

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The laying bird is characterized by an exceptionally high phosphorus turnover. Not only does phosphorus leave the bird through the usual channels of excretion but, in addition, large amounts are deposited in the egg. A single 60 gram egg may contain as much as 0.5 gram of phosphorus combined in various compounds.

The radioactive isotope of phosphorus provides a useful tool for tracing the passage of phosphorus into the egg. In the present study, the rate of appearance of labeled phosphorus was followed in the shell, membrane, albumen and yolk.

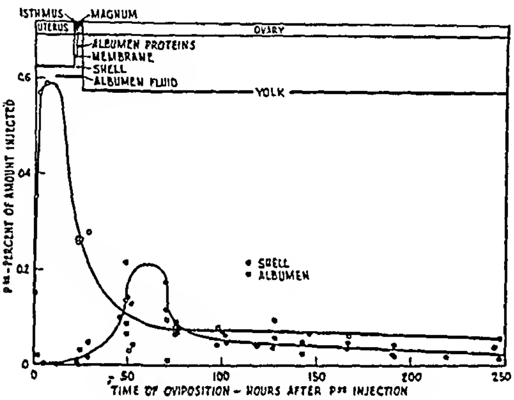
Experimental. Six laying White Leghorn hens (Gallus domesticus) were maintained in individual cages equipped with glass dropping trays. Each bird received subcutaneously 1.5 cc. of an isotonic solution of radioactive NaH_2PO_4 containing 10 microcuries of P^{32}. At the same time 0.5 cc. of an emulsion of Sudan III, similar to that used by Warren and Conrad (1), was injected intravenously. Some of this fat-soluble dye was deposited at once in the developing ova, forming a shell 0.5 mm. thick, clearly marking the size of the yolk at the time the radioactive phosphorus was injected. Droppings were collected at 6, 12 and 24 hours after the injection and at 24-hour intervals thereafter. These droppings were air-dried, ground and ashed. Eggs were collected at hourly intervals. They were frozen and then cut through the center of the yolk. Diameters of colored rings were measured. Shell, membrane, albumen and yolk were separated. An aliquot of the yolk was stored in alcohol for isolation of phospholipids; the remainder of the yolk and other parts of the egg were ashed in the presence of nitric acid and magnesium nitrate. For the determination of phospholipid the samples were extracted and mounted in the manner previously described (2).

Results. The early growth of the ovum is a slow process (1, 3). Several months are required for the ovum to attain a diameter of 6 or 7 mm.; during this time only white yolk is deposited. The final stage of yolk growth, which involves the deposition of yellow yolk, is a much more rapid process, requiring 7 to 10 days for completion. During the first part of this period the yolk diameter increases about 2 mm. per day, but during the last 3 or 4 days the daily increment in yolk diameter gradually decreases.

Within a few minutes after ovulation the yolk is picked up by the infundibulum of the oviduct. It traverses the magnum in 3 hours and is meanwhile surrounded with concentrated albumen (fig. 1). The egg obtains all of its albumen protein and about half of its water while traversing this structure. It

next enters the isthmus. It requires a little over an hour for its passage through here, and during this interval the egg receives its membranes. During its stay in the isthmus some water and crystalloids enter the albumen by osmosis through the membranes. The egg next enters the uterus, where it remains, on an average, 21 hours. During this interval the shell is deposited; additional water and salts are also added to the albumen during the early part of this interval.

Deposition of labeled phosphorus in egg shell. Labeled phosphorus was found in the shells of all eggs laid subsequent to P^{32} injection. The period of observation extended over an interval of 504 hours. One egg present in the uterus at the time of P^{32} administration was laid one hour later, and its shell already contained 0.15 per cent of the injected P^{32}. Since about 21 hours are required



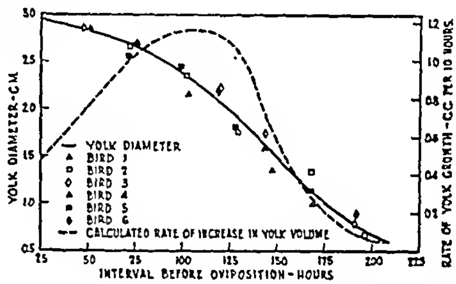


Fig. 1 Fig. 2

Fig. 1. Labeled phosphorus deposition in albumen and shell. Each point represents the quantity formed in the whole albumen or shell of an egg laid at time indicated. The position of the egg in the bird's reproductive tract at the moment of P^{32} injection is shown at the top.

Fig. 2. Increase in yolk size. Solid line represents yolk diameter at time of P^{32} injection as measured by dye technique. Broken line shows rate of increase of yolk volume calculated from yolk diameter curve.

to form a shell, it is apparent that very little could have been added to the shell in the last hour. Three other eggs, laid, 2, 3 and 6 hours after injection, contained progressively larger amounts—namely, 0.35, 0.57 and 0.59 per cent respectively. The rapidity with which labeled phosphorus appeared suggests that blood phosphorus is immediately available for deposition in the shell.

The next eggs were laid 24 to 30 hours after the injection of labeled phosphorus. Shell formation was therefore not initiated in these eggs until 3 to 9 hours after the P^{32} injection, and so P^{32} was available during the entire period of shell formation. Yet these shells contained only 0.23 to 0.28 per cent of the injected P^{32}, amounts even less than that present in the shell of the egg laid only 2 hours after injection. Succeeding egg shells had even smaller amounts of phosphorus, but the quantity decreased only very slowly in shells of eggs that were laid after 50 hours after injection. In figure 1 values for shell P^{32} are plotted against the

interval between the injection and the time the egg was laid. Values for intervals up to 250 hours only are shown in figure 1. Radioactivity, however, was still perceptible in the shell of an egg laid as late as 504 hours.

As pointed out above, only those shells that were being actively formed at the time of the injection showed a high P^{32} content, whereas eggs that entered the uterus several hours later contained much smaller amounts of P^{32} in their shells. This indicates that the source of shell phosphorus is a rapidly exchanging entity, presumably inorganic phosphorus.

In contrast to these findings on egg shell, no significant radioactivity was detected in the shell membrane at any time.

Radiophosphorus deposition in the albumen. Since albumen protein is secreted while the egg is in the magnum and its deposition is completed about 22 hours before oviposition, protein-bound radiophosphorus could not have appeared in eggs laid less than 22 hours after P^{32} injection. Some crystalloidal P^{32} might, however, have been added to eggs laid as early as 12 hours after its administration. Nevertheless, no appreciable quantities were found in albumen of eggs laid during the first 24 hours (fig. 1).

Eggs laid during the 24–30 hour interval entered the magnum at a time when blood radiophosphorus was probably at a maximum as judged by P^{32} deposition in the shell. These eggs, however, did not contain the greatest amounts of albumen radiophosphorus. As shown in figure 1, the P^{32} content in the albumen of these eggs did not exceed 0.05 per cent, whereas eggs laid between 45 and 75 hours contained 2 to 4 times this amount. Albumen of succeeding eggs showed low levels of P^{32}, which continued to decrease throughout the period of observation. Radioactivity was still perceptible in the last egg that was analyzed; this egg was laid 504 hours after injection.

There is thus a marked dissimilarity in the deposition of phorphorus in shell and in albumen. The delayed deposition of P^{32} in the albumen suggests that a synthetic process precedes the deposition of phosphorus-containing compounds. Since at least one albumen protein, namely, ovalbumin, is known to contain phosphorus (4), the delay is not unlikely due to the incorporation of phosphorus into this or other protein before their deposition in the albumen of the egg.

Radiophosphorus deposition in egg yolk. Yolks of eggs laid less than 24 hours after injection contained only traces (up to 0.0045 per cent) of the administered P^{32}. Since an egg spends 24 to 25 hours in the oviduct after ovulation, these yolks had already completed their growth and had left the ovary at the time the injection was made. Consequently, no P^{32} was to be expected except for such small amounts as may have passed through the vitelline membrane by osmosis from the albumen. Two birds (2 and 5) laid eggs 30 hours after injection, and yolks of these eggs each contained only 0.010 per cent of the administered phosphorus. The next eggs obtained were from bird 3 at 47 hours, bird 4 at 50 hours, and bird 1 at 51 hours. Yolks from these eggs had been in contact with the ovarian vascular system for 22 to 26 hours after the injection of P^{32}; these contained appreciably larger amounts of the labeled phosphorus (0.275, 0.985 and 0.605 per cent respectively).

The quantity of P^{32} in yolks of succeeding eggs laid by each bird increased until a maximum was reached; the maxima for all birds were observed between 98.5 hours (bird 5) and 146.8 hours (bird 1) after the injection of P^{32}.[1] The maximum quantities of radioactive phosphorus varied from 1.19 per cent for bird 89 to 2.48 per cent for bird 5. Following maximum deposition, the amounts of radiophosphorus in succeeding yolks decreased abruptly at first and more gradually thereafter.

A simple examination of such data affords little information about the factors involved in the deposition of a particular substance in the egg yolk. One of the complicating factors is the rapid change in the amount of P^{32} available for yolk formation. Thus, yolks proceeding through a particular phase of growth several days after the P^{32} is administered will necessarily draw upon phosphorus of lower specific activity than those yolks passing through the same growth phase at an earlier time. Moreover, in view of the marked differences in the types of P compounds occurring in yolk, it would be desirable to consider the deposition of individual phosphorus compounds rather than total phosphorus.

In the present study attempts have been made to resolve these difficulties as follows: 1. Egg yolk phosphorus was separated into two components: phospholipid and non-phospholipid or "residual" phosphorus. This separation, of course, merely divides the phosphorus present into two main groups. 2. From theoretical considerations, attempts were made to calculate a series of yolk P^{32} values that agreed closely with the experimentally observed values of P^{32} in each yolk fraction. The best agreement was obtained when values were calculated on the assumption that the amount of the P^{32} fraction in any small increment of newly deposited yolk is proportional to the concentration of that P^{32} fraction or its precursor in the circulation at that time. The theoretical expression thus obtained (see addendum) permitted calculation of a single series of values for each phosphorus fraction that agreed well with the distribution of experimental values in successive yolks from the same bird (figs. 4 and 5). The theoretical expression differed from the experimental values by a factor which, though constant for all values of a single bird, differed for different birds.

Radiophosphorus excretion. In marked contrast to the patterns of P^{32} deposition observed in different parts of the egg, excretion of radiophosphorus in the droppings (mixed urine and feces) showed a marked irregularity. The hourly excretion of radiophosphorus in the droppings of individual birds is plotted in figure 6. In each histogram, the abscissa represents the period during which the sample was collected, and the ordinate represents the average hourly excretion during that period. The arrows represent periods during which egg shells were formed, and each point of the arrow shows the time the egg was laid. The four rapidly laying birds, 1, 2, 4 and 5, excreted radiophosphorus slowly and somewhat irregularly. Except for a somewhat higher excretion rate during the

[1] The maximum value for bird 6 was 0.651 per cent in an egg laid 76 hours after injection; this egg, however, was followed by a double-yolked egg at 118.8 hours, the two yolks of which contained 1.49 per cent. To avoid complicating factors this latter egg has been eliminated from discussion.

first day, variations were not great and seemed to occur at random. By contrast, however, the two slowly laying birds, 3 and 6, showed periods of increased excretion that coincided with periods of shell formation.

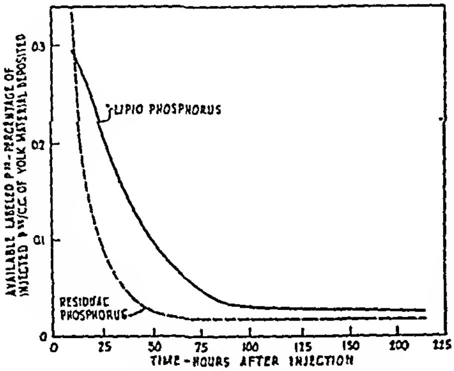


Fig. 3

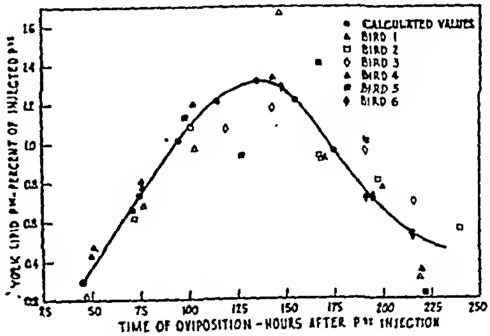


Fig. 4

Fig. 3. Availability of P^{32} for lipid phosphorus and residual phosphorus of yolk. Curves chosen by trial and error to fit observed P^{32} data.

Fig. 4. Labeled phospholipid content of yolks of eggs laid after P^{32} injection. The curve was calculated from equation (1). Each point represents total radiophospholipid in one yolk, adjusted so that value for the yolks from each bird have the same mean as corresponding points on calculated curve. (Points from each bird are thus plotted as deviates from a single theoretical curve. To obtain actual values, multiply ordinates of curve by corresponding factor recorded in table 1 and add indicated deviate. For example, to obtain actual value for bird 41 at 103 hours, multiply 1.10 (read from curve) by 0.694 (from table 1) and subtract 0.13 (which is the difference between the plotted point and 1.10).)

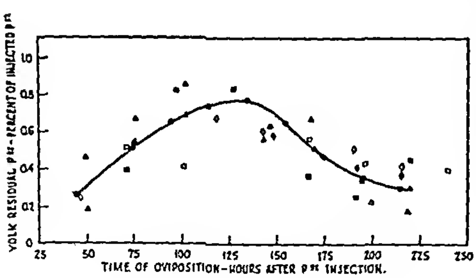


Fig. 5

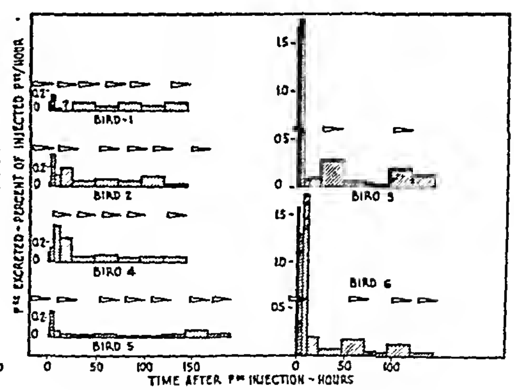


Fig. 6

Fig. 5. Residual radiophosphorus content of yolks of eggs laid after P^{32} injection. Calculated curves and experimental deviates plotted as in figure 4. For meaning of symbols see figure 4.

Fig. 6. Rate of labeled phosphorus excretion in the droppings. Arrows point to time of oviposition and extend over period egg remained in uterus.

These results are particularly interesting in view of the findings of R. H. Common (6, 7, 8), who reported that phosphorus excretion in the droppings increased during egg-shell formation in birds on a diet containing 0.67 per cent

calcium or less, but not as much as 2.21 per cent calcium. The birds in th present experiment were maintained on a diet containing 1.21 per cent calcium The pattern of P^{32} excretion in only 2 of the 6 birds studied was similar to tha demonstrated by Common for total phosphorus. These results are not neces sarily contradictory, however, for the two birds that followed Common's patter were slow-laying, as were all the birds studied by him.

Discussion. Although the mechanism proposed to account for the phos phorus deposition in yolk (namely, that the total amount present is an integra of the product of yolk-growth rate and phosphorus availability[2]) was found t fit the experimental data satisfactorily, one obvious discrepancy was left un resolved. Large individual differences among birds necessitated the use o different factors (Table 1) in order to compare the theoretical curves wit experimental values. These factors (which correspond to k in equation 1 varied between 0.39 and 1.41 for the radiophospholipid curve and between 0.5 and 1.27 for the residual P^{32} curve. The underlying nature of such factor deserves inquiry. The two lowest residual phosphorus factors were observe for birds 3 and 6, and the lowest and third lowest phospholipid factors were als

TABLE 1

k values for equation (1)

BIRD	PHOSPHOLIPID P^{32}	RESIDUAL P^{32}
1	0.694	1.274
2	1.367	0.959
3	0.793	0.574
4	1.166	1.142
5	1.414	1.135
6	0.391	0.634

found in these birds. These 2 birds were the slow layers that excreted larg amounts of phosphorus in their droppings.

This relation between rate of laying, phosphorus excretion, and the above k values offers an explanation of the low factors of birds 89 and 92 and thereby extends Common's theory. Common explained his results by postulating a withdrawal of calcium phosphate from the skeleton for shell formation, the calcium being used for the shell and the extra phosphorus excreted. Evidence confirming this view was offered by Feinberg, Hughes and Scott (9), who demonstrated an increased blood inorganic phosphorus during the time of shel formation (from 4.2 mgm. per cent immediately after ovulation to 7.5 mgm per cent after the egg reached the uterus). The phosphorus availability curves in figure 3 then must be partly dependent on the presence or absence of eggs in the uterus, and should show corresponding fluctuations. No attempt was made to include these fluctuations in figure 3, and they probably account for part of the experimental error (equation 4), although the method of treatment was such as to minimize errors due to such fluctuations. A gross difference

[2] See addendum.

between the two slow-laying and the four rapid-laying birds, however, cannot be neglected. In the former, shells were being produced less than 30 per cent of the time during the first 100 hours after injection of P^{32}, and in the latter about 80 per cent of the time. This difference must have been responsible for a large gross difference in the phosphorus-availability curves.

Shell formation, as already noted, presumably calls forth calcium and phosphorus from bone. The ratio of calcium to phosphorus in shell is much higher than in bone; obviously, therefore, the extra phosphorus must be disposed of elsewhere than in the shell. In the fast-laying birds, the rapidly growing ova have access to this extra phosphorus. The ovaries of the slow-laying birds, by contrast, probably contained relatively few rapidly growing yolks which could take up only a small portion of the extra blood phosphorus when made available. Consequently, in conformity with the pattern described by Common, a large portion of this extra phosphorus appeared in the droppings of the slow-laying birds as "extra" excretion during shell formation.

In the preceding discussion the assumption has been made that the availability curves (fig. 3) bear a close relation to the true plasma labeled phosphorus curves. Such an assumption will be valid only if the action of the follicular membrane does not change during the period of yolk growth considered here, in respect to any selective action it may exert on phosphorus-containing compounds supplied to it by the plasma. The data available do not permit an estimate of the shape of the portions of the availability curves during the first ten hours after injection. Certain general properties of the curves in this region are obvious, however, from the shapes of the curves as drawn. Both curves must have a maximum within ten hours after the injection, and the maximum for the residual phosphorus curve probably occurs earlier and is higher than that for the phospholipid curve.

Data on the deposition of radiophosphorus in the egg have appeared in three previous communications (10, 11, 12). Entenman et al. (10) injected P^{32} and determined labeled phospholipid deposition in growing ova during an interval of six hours after injection. Radiophospholipid deposited in smaller ova, which weighed between 0.69 and 5.8 grams, varied from 0.0023 to 0.058 per cent, increasing regularly with increasing size of the ovum. In larger ova this relation no longer held; the maximum value, 0.068 per cent, was found in a 10.7 gram ovum, and ova larger than this had somewhat smaller quantities of radiophospholipid. A similar distribution of values, although with correspondingly larger total quantities, was obtained from ova of birds killed twelve hours after P^{32} injection. Such values fit well with expected deposition rates that might be predicted from the yolk growth curve in figure 2. This correspondence may be extended by the two values obtained in the present investigation for yolks in which radiophospholipid was deposited for five to six hours after P^{32} injection at the end of their growth period. These yolks received only 0.010 per cent of the injected P^{32} as phospholipid, an amount very much less than that found in the largest ovum taken from the ovary.

Hevesy and Hahn (11) measured radiophospholipid deposition in the smaller

ova of a single hen killed 28 hours and two ova from another bird killed five hours after P^{32} injection. The results, expressed as "specific activity," cannot be compared directly with the foregoing, but nevertheless demonstrate the same increase with increasing size of smaller ova. These investigators also measured P^{32} distribution in five eggs laid subsequent to P^{32} injection. All of their values were appreciably lower than those reported in the present paper at comparable intervals. From the low activities found in the albumen of the first egg laid (reported variously as 4.5 hours and as 0.17 day) after injection and in an egg removed from the oviduct of another bird 5 hours after injection, they postulate a delay in phosphorus-compound formation similar to that noted here. The 4.5 hour egg, of course, must have been in the uterus with its shell over half formed at the time of injection; consequently the P^{32} found must have diffused through both shell and membrane with the last bit of fluid that is added to the egg in the uterus. The second and third eggs measured by these investigators were laid 24 and 72 hours after injection, and therefore they missed the deposition peak recorded here.

The radioactive phosphorus used in this investigation was supplied by members of the Radiation Laboratory under the direction of Prof. E. O. Lawrence, to whom our thanks are due.

SUMMARY

1. Injected phosphorus makes its appearance rapidly in the shells of eggs. Largest amounts were found in shells being formed at the time of P^{32} injection.

2. The deposition of labeled phosphorus in albumen was delayed. Maximum deposition of P^{32} occurred in eggs in which albumen was actively secreted 24–54 hours after the P^{32} injection.

3. Administered P^{32} makes its appearance rapidly in the phospholipid and other phosphorus compounds of the yolk. The amount of P^{32} deposited in these fractions in the yolk could be accounted for by an integral function of the two variables, yolk growth rate and P^{32} availability, during the corresponding period of new formation; values so calculated agreed well with observed values. The availability curves for yolk phosphorus and yolk phospholipid have been roughtly defined.

4. A faster deposition of P^{32} was observed in yolks of fast-laying than in slow-laying birds.

5. Excretion of injected P^{32} is shown to depend upon *1*, rate of egg production, and *2*, whether shell formation is in progress.

Addendum. The deposition of a P^{32} fraction in yolk may be considered to take the form:

$$y = k \int_{t_i}^{t_o} x \frac{dv}{dt} dt \tag{1}$$

where y is the total quantity of the substance containing P^{32} found in any one egg yolk, x a function of the amount of P^{32} available for the formation of substance y at time t, and $\frac{dv}{dt}$ the rate of growth of the yolk. The integration is carried out over the interval from t_i,

the time of injection, to t_o, the time of ovulation. Neither x nor $\frac{dv}{dt}$ is known analytically, but

$$\frac{\Delta v}{\Delta t} = f(t) \tag{2}$$

can be obtained graphically from the data shown in figure 2. The general form of the curve

$$x = \phi(t) \tag{3}$$

can be approximated from theoretical considerations. With an experimental curve for the one function (equation 2) and a hypothetical curve for equation 3, it should be possible to perform the integration of equation 1 graphically, to obtain a curve that corresponds closely to the experimental curve for the egg yolk phosphorus component (y), and to test the fit statistically.

To obtain the curve for $\frac{\Delta v}{\Delta t} = f(t)$, use was made of rings obtained in the yolks by Sudan III injection at the time of radiophosphorus administration. The yolk diameters at the time of injection so obtained were plotted against the interval between injection and oviposition (fig. 2) and a mean curve was drawn. From values read off this mean curve, volume increments were calculated by assuming that yolks are perfect spheres. Ten-hour volume increments were plotted against time before oviposition (fig. 2).

To obtain an approximation to the curve $x = \phi(t)$ it was assumed that the labeled phosphorus, after reaching an early maximum in the bloodstream by rapid absorption from subcutaneous tissues, is lost from the blood at a rate proportional to the amount present. This relation could hold, however, only in the earlier stages; instead of approaching zero asymptotically, the available phosphorus should remain at a low but very slowly decreasing level for a long time, since labeled phosphorus deposited earlier in the skeleton gradually re-enters the circulation. Cook, Scott and Abelson (5) have shown that chicken bones contained appreciable amounts of radiophosphorus 75 days after injection. Such a curve should bear a close similarity to the experimental curve of radiophosphorus deposition in shells, since the amount of P^{32} deposited in the shells responds most readily to the level of circulating P^{32}. Starting with this curve as a first approximation to the P^{32} availability curve, two curves were finally chosen by trial and error to represent availability of P^{32} for phospholipid and residual P. These curves are shown in figure 3.

A theoretical curve for deposition of a phosphorus compound could then be obtained by integrating equation (1) graphically for a sufficient number of points. Nine such points (each representing a hypothetical yolk) were chosen to define the curve between oviposition intervals of 45 to 215 hours after injection of P^{32}. Each point was obtained by summing products of values read from figure 2 ($\frac{\Delta v}{\Delta t}$ values) and from figure 3 (x values) at corresponding 10-hour intervals between the time of injection and of ovulation of the hypothetical yolk. To be sure, the points do not define a unique curve, but rather a series of curves, since the nature of k (equation 1) has not been considered. As stated above, k differs for different birds. Thus to compare this curve with the experimental data the graphically integrated function $\Sigma(x \frac{\Delta v}{\Delta t})$ must be multiplied by a different k for each bird. Such curves were drawn for each bird, and the deviates (d_i) of each experimental point were thus obtained from the individual theoretical curve for that bird (table 1). To save space, however, a single theoretical curve only was plotted for each fraction (phospholipid-P^{32} in fig. 4; residual-P^{32} in fig. 5), with the average value of k. How well the experimental points fit their individual theoretical curves is illustrated by plotting their deviates (d_i) on the mean theoretical curve.

How well the experimental points fit the theoretical curves was tested by calculation of t values for various regions; a significant t value would indicate a significant departure of the points from the theoretical curve and, hence, a poor fit in that region. Deviates from the individual theoretical curves as previously defined were used to calculate t for each region by the formula:

$$t = \frac{\sum^{n} d_i}{\sqrt{n}} \sqrt{\frac{N - 6}{\sum^{N} d_i^2}} \tag{4}$$

where the values of d_i of all birds in the region under test are summed. N is the total number of observed values and n the number of observations in this region.

For test the curves were divided into four approximately equal segments; the t value for each region was non-significant. In one region only (100–149 hrs. on the residual phosphorus curve) was the probability as low as 0.1; in all other instances the probability varied from 0.4 to greater than 0.9. This test becomes more sensitive as shorter and shorter regions are tested, and may even be applied to individual points. In this test only a single point on the residual phosphorus curve and only 2 points on the phospholipid curve had t values large enough to be assigned a probability of 0.05, the accepted minimum criterion of significance. Further, the deviates can be shown to be normally distributed. The theoretical curves, therefore, may be said to fit the experimental points adequately, and the hypothetical mechanism of radiophosphorus deposition (equation 1) may serve as a first approximation, at least, to the actual mechanism.

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OXYGEN CONSUMPTION IN VITAMIN E DEFICIENCY[1]

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Vitamin E deficiency leads to extensive degeneration of the skeletal muscles in several species of mammals. Previous studies in rabbits, guinea pigs, older rats, and hamsters by Victor, Madsen, Friedman and Mattill, and Houchin have shown an increase in the *in vitro* O_2 consumption of the muscles (1, 2, 3, 4). These studies, however, have been carried out in animals in which dystrophic changes in the muscles had taken place and in which the interpretation of the results may have been complicated by the presence of inflammatory and regenerating cells or by the replacement of the muscle fibers by fat or connective tissue. Moreover, in the experiments of Victor, Madsen and Friedman and Mattill, the data were compared with those obtained from animals on a stock diet rather than with those on an experimental one supplemented by alpha-tocopherol.

It seemed important, therefore, to determine whether a rise in the rate of O_2 consumption of the rat muscle *in vitro* occurred also in the absence of morphologic changes, and whether administration of alpha-tocopherol would prevent the increase.

We have also included in our studies muscles from a species in which the manifestations of vitamin E deficiency are chiefly limited to the central nervous system. This is the case in chicks (5). With the vitamin E deficient diet used in this laboratory (diet 108), the skeletal muscle is rarely affected and only in animals showing the "alimentary exudative diathesis" described by Dam, Glavind, Bernth and Hagens (6).

Information as to the O_2 consumption of whole animals kept on a vitamin E deficient diet is scant. Wood and Hines (7) reported 116 examinations on 10 guinea pigs on a vitamin E deficient diet which were examined at weekly in-intervals over a period of 2 to 3½ months. No significant difference between these animals and controls on a stock diet could be discovered when the values were expressed in cubic centimeters of O_2 per hour per square meter.

Telford, Emerson and Evans (8) found in preliminary studies on 4 vitamin E low and 4 normal rats (whose age is not stated) no significant differences. The average figure for the vitamin E deficient animals was "slightly higher" than that for the normal ones.

Biddulph and Meyer (9) examined the O_2 uptake of rats which had been kept on a vitamin E deficient diet after the age of one month. They found the O_2 consumption in vitamin E deficient animals significantly higher than that of rats on a stock diet. This difference, however, did not become evident until after six months on the experimental diet, and was associated with enlargement

[1] Aided by a grant from the John and Mary R. Markle Foundation.

of the thyroid gland. Addition of KI to their diet decreased both the oxygen consumption and the thyroid weight, but the administration of wheat germ oil alone had the same effect.

These studies and those of the other workers cited did not include observations on young rats during the acute phase of muscle degeneration when the lesions are most severe, nor upon the possible effect of the administration of alpha-tocopherol. It seemed important to find out whether the increase in O_2 consumption noted in excised muscle might be reflected in an increased O_2 uptake of the living animals and, if so, to what extent this could be prevented by the administration of tocopherol.

I. O_2 CONSUMPTION OF RAT AND CHICKEN MUSCLE IN VITRO. 1. *Rats.* The mother animals were maintained from the time of weaning on a vitamin E deficient diet which consisted of: casein (commercial), 320 grams; corn starch, 400 grams; salts (Hawk and Oser), 40 grams; yeast (baker's dried), 100 grams; lard, 220 grams; cod liver oil, 20 grams. During lactation, 100 grams of yeast were added to the diet.

When mating was positive, the litter was assured by protecting the mother with 50 drops of wheat germ oil given within five days after mating. Under these conditions about 90 per cent of the offspring develop lesions (10).

The young in which protection was desired received one drop of sesame oil, containing one milligram of synthetic dl-alpha-tocopherol acetate (Hoffmann-La Roche)[2] by mouth on the 15th day. They were separated for a certain time from the unprotected animals in order to make the alpha-tocopherol inaccessible to them. Muscles from rats maintained on a stock diet (Rockland pellets) were also studied for comparison.[3]

Chicks. These were placed 3 or 4 days after hatching upon the standard encephalomalacia-producing diet 108 (11). Dl-alpha-tocopherol acetate was given in daily doses of 0.2 mgm. in gelatin capsules. Control chicks were given commercial mash (Ful-o-Pep Egg Breeder Mash made by the Quaker Oats Company).

The O_2 consumption was measured in Fenn's constant pressure differential manometer (12). The modification described by Victor and Potter (13) was followed in this work. QO_2 is expressed in millimeters^3 per gram wet weight in 1 minute. The water bath in rat experiments was kept at 37°C. and in the chick experiments at 39°C.

The animals were killed by decapitation. The flexor muscles of the hind legs and the pectoralis muscles were chosen for study in rats; in chicks, only pectoralis muscle was used. The pieces were sliced into thin strips with a safety razor blade while still *in situ*. About 15 minutes elapsed between the death of the animal and the immersion of the bottles with the muscle fragments

[2] We are greatly indebted to Dr. R. D. Shaner of the Hoffmann-La Roche Company for supplying dl-alpha tocopherol acetate.

[3] The Rockland diet is stated to contain 3.2 mgm. per cent of alpha-tocopherol as determined by rat bio-assay. We are indebted to Dr. Charles A. Slanetz of the Department of Animal Care for this information.

in the water bath. For each examination the average of at least two control bottles was taken. In most of the experiments litter-mates were examined on the same day.

Histological sections of the muscles were made from all animals and in addition, the muscle pieces used for the determination of the oxygen consumption were fixed in 10 per cent formalin at the end of the experiment and examined histologically.

Experiments. *Rats.* The individual data on leg muscles of rats, together with the mean, are shown in figure 1, and the results of all experiments are summarized in table 1.

The mean O_2 consumption of the leg muscle from the vitamin E deficient group during the first hour is significantly greater than that from the group which received a single dose of dl-alpha-tocopherol acetate given between the

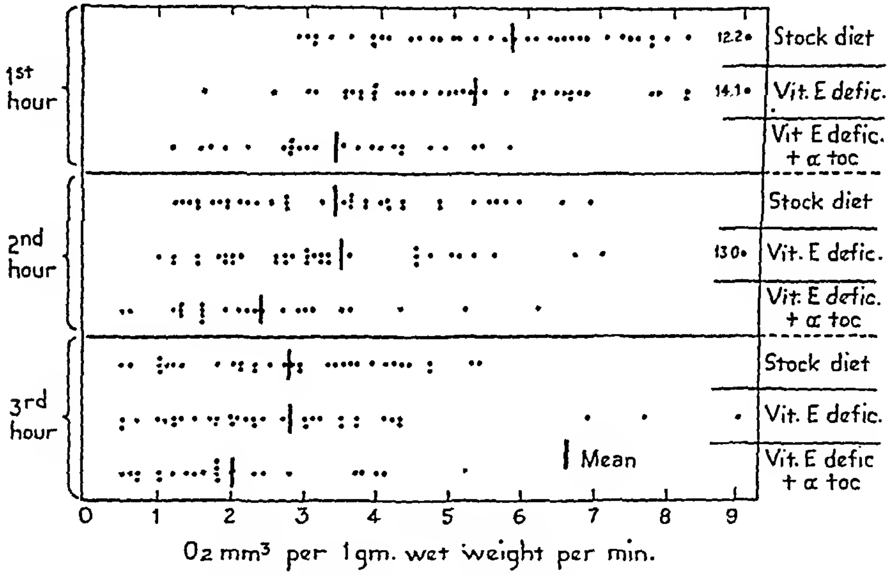

Fig. 1. O_2 consumption of excised rat muscle from rats on experimental diets

13th and 15th days. The difference is also significant during the second hour, but tends to disappear in the third hour after the beginning of the experiment.

Comparing the vitamin E deficient animals with those on stock diet, presumably containing an abundance of vitamin E, it is surprising that there is no statistically significant difference in the O_2 consumption. Stock animals which received a supplementary single dose of 1 mgm. of dl-tocopherol acetate showed practically the same values. While we are unable to account for this discrepancy, the difference in the composition of the stock diet and the simplified experimental diet makes it possible that other factors than the vitamin E content may be involved.

The data obtained from the pectoralis muscle show differences in the same direction between the muscles of the vitamin E deficient group and those which had received a protective dose of alpha-tocopherol. The difference is manifest only during the first hour and is less pronounced than with the leg muscles.

Table 2 shows that there is no statistically significant difference between the O_2 consumption of muscles showing dystrophic lesions and those which were histologically normal.

In order to ascertain whether other tissues than the skeletal muscle show an increased O_2 consumption in vitamin E deficiency, determinations were made on the liver of 19 vitamin E deficient, and 17 tocopherol treated rats. No significant difference was found.

Chicks. A summary of our determinations is given in table 3. The experiments were run in two series—one in the spring of 1941 and the other in November and December of the same year. There was no indication of a seasonal difference, and the data show close agreement.[4]

TABLE 1

In vitro O_2 consumption of rat muscles

(Cubic millimeters per gram wet weight per minute)

TISSUE	AGE RANGE	DIET	NO. OBS.	FIRST HOUR			SECOND HOUR			THIRD HOUR		
				Q/O_2	Range	σ	Q/O_2	Range	σ	Q/O_2	Range	σ
	days											
Hind leg muscle	19–26	Stock	38	6.8	3.9–12.2	1.83	4.4	2.2– 7.9	1.56	1.56	1.5– 6.4	1.36
Hind leg muscle	19–20	Stock + single dose of 1 mgm. α toco-	13	6.4	3.3– 8.4	1.49	4.8	2.0– 6.8	1.28	4.3	1.2– 6.5	1.46
Hind leg muscle	14–23	Vitamin E deficient	37	6.3	2.6–14.1	2.10	4.5	2.0–13.0	2.19	3.8	1.5–10.0	1.92
Hind leg muscle	14–23	Vitamin E deficient + single dose of 1 mgm. α tocopherol	24	4.4	2.2– 6.8	1.29	3.4	1.5– 7.2	1.36	3.0	1.5– 6.2	1.31
Pectoralis muscle	14–23	Vitamin E deficient	23	5.7	2.8– 8.8	1.46	3.8	2.2– 6.5	1.27	3.0	1.5– 6.4	1.36
Pectoralis muscle	14–23	Vitamin E deficient + single dose of 1 mgm. α tocopherol	24	4.9	2.9– 8.3	1.15	3.8	2.1– 5.7	1.10	3.2	1.5– 5.9	1.15

Discussion. The above findings confirm the previous observations of Victor,

[4] Dr. Joseph Victor and Dr. L. L. Madsen very kindly put at our disposal unpublished data on O_2 consumption of chick muscle. Their determinations were carried out in this laboratory several years ago, but their data are not strictly comparable to ours, inasmuch as the metabolism of the muscle of vitamin E deficient chicks in their experiments was compared with that of chicks on a simplified complete diet, but not on diet 108 supplemented by alpha-tocopherol. Both these workers, however, found a higher oxygen consumption on the vitamin E deficient diet—a result in line with our own observation.

Madsen, Friedman and Mattill and Houchin as regards the increased uptake of oxygen in dystrophic muscles. Our experiments, however, bring out the significant point that this metabolic change may be detected in rats even when the E deficient diet has brought about no anatomical changes in the muscle, and in chicks in which the skeletal muscle is unaffected. After dystrophic lesions are established, there is always a reaction in which wandering cells and young connective tissue cells are concerned. The total oxygen consumption under these conditions represents the composite activity of several tissue elements. In our material, excluding the 11 cases in which lesions had developed, the increased oxygen consumption must be referred to a change in the metabolic activity of the muscle fiber itself.

TABLE 2

In vitro O_2 consumption of rat muscle showing dystrophic lesions on a vitamin E deficient diet

(Cubic millimeter per gram wet weight per minute)

LEG MUSCLE			PECTORALIS MUSCLE		
First hour	Second hour	Third hour	First hour	Second hour	Third hour
7.7	5.5	4.5	5.0	2.7	2.1
4.6	2.8	2.3	4.3	2.2	1.5
9.2	8.1	8.7	8.8	6.4	6.4
5.9	4.0	3.0	5.0	3.9	2.6
5.6	3.7	3.3	5.3	2.9	1.7
7.1	6.3	4.7	7.1	5.3	5.2
6.1	5.5	5.2			
5.4	2.5	3.2			
4.1	3.8	4.0			
4.7	3.1	2.0			
2.6	2.5	2.2			
Mean..5.7	4.3	3.9	5.9	3.9	3.3
σ......1.81	1.79	1.96	1.70	1.65	1.92

TABLE 3

In vitro O_2 consumption of chick pectoralis muscle

(Cubic millimeter per gram wet weight per minute)

DIET	NO. OBS.	FIRST HOUR			SECOND HOUR			THIRD HOUR		
		Mean	Range	σ	Mean	Range	σ	Mean	Range	σ
Stock...............	18	5.4	2.7–9.1	1.72	3.5	1.2–5.9	1.19	2.5	1.1–4.5	0.92
Vitamin E deficient.	27	6.1	2.8–9.9	1.12	4.2	2.4–7.0	1.22	3.7	2.2–5.8	0.92
Vitamin E deficient single dose of 1 mgm. α tocopherol	39	5.3	2.7–9.3	1.41	3.7	1.2–5.3	0.99	3.1	1.3–4.4	1.83

It is further apparent from our data that the administration of synthetic

alpha-tocopherol acetate in a dose of one milligram given on the 11th to 14th day after birth significantly lowers the oxygen consumption as compared with the untreated controls. Friedman and Mattill (3) have reported a few preliminary experiments on 5 months old female rats in which the administration of 5 mgm. of alpha-tocopherol acetate lowered the oxygen consumption after 24 and 72 hours respectively.[5]

An important question, which has not yet been investigated, is whether the increased oxygen consumption is specifically limited to skeletal muscle or whether it is a general effect on the tissues. The fact that the increased oxygen consumption was not found in the liver suggests a characteristic effect on the muscle metabolism. Further studies, however, on other tissues would be needed to demonstrate this specificity.

II. Total oxygen consumption. Three groups of rats were used; the first received stock diet, the second the vitamin E deficient diet, the third the vitamin E deficient diet supplemented by a single dose of 1 milligram dl-alpha-tocopherol acetate on the 15th day of life. The tocopherol was given by mouth in a drop of sesame oil.

The animals were placed in the apparatus after a fasting period of one hour; the tests were performed about one hour later. They were purposely not fasted over a longer period because it was necessary to examine the young ones daily or every other day.

The O_2 consumption was determined in a closed circuit apparatus, the construction of which is shown in fig. 2 a, b, c. The apparatus is a modification of that described by Rappaport and Gottdenker (14). Single tests of 5 to 10 minutes' duration can be carried out with sufficient accuracy; in fact, the short duration of a single experiment is an advantage for the determination of the O_2 consumption of small animals, inasmuch as they can be examined in periods of complete quietness.[6]

After the apparatus is adjusted and tested, the actual procedure is as follows. The syringe is put into motion; the animal is placed in the container; care must be taken that the three-way stopcock permits free communication between the outside air and the apparatus, so that the O_2 consumed by the animal in the preliminary period can be instantly replaced. After about 30 minutes, the float of the spirometer is elevated and the three-way stopcock is put into a position in which it connects the spirometer with the apparatus and closes off the outside air. Several curves are taken, and only entirely straight ones, indicating the absence of movements of the animal, are computed. The lowest value (not the

[5] Since the completion of these experiments, there has appeared a paper by Houchin and Mattill (Proc. Soc. Exper. Biol. and Med. 50: 216, 1942), demonstrating an increased *in vitro* O_2 consumption of dystrophic hamster and rabbit muscle. The direct addition of water soluble alpha-tocopherol phosphate to the Locke-Ringer solution reduced the O_2 uptake by 35.7 per cent and 41.3 per cent—figures which roughly correspond to the difference found by us between vitamin E deficient rat muscle and muscle from animals which had received a supplementary dose of tocopherol before death.

[6] Because of the need to economize space, details as to calibration and testing of apparatus are omitted.

average) is eventually used, as it most closely expresses the resting metabolism.

Measurements were made at a temperature of 29°C. and the rats were permanently maintained at a temperature of 25 to 29°C. in order to obtain stable and comparable results (Benedict and Macleod, 15). The rats were weighed immediately after the test.

The determinations began on the 10–14th day of life and were carried out on the same animal during the first 3 weeks, daily or on every other day, later once a week. Litter-mates were usually examined on the same day. In order to avoid diurnal variations (Horst, Mendel and Benedict, 16), tests were performed only between 10 a.m. and 4 p.m.

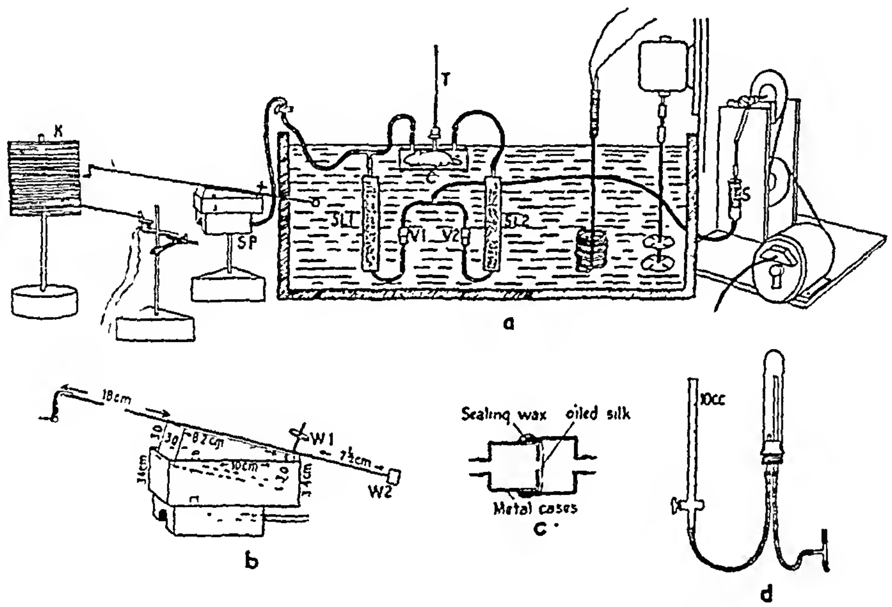

Fig. 2a. Closed circuit apparatus. *C*, animal container, dimensions 20 x 8 cm., closed by threaded glass windows. *SL 1* and *SL 2*, soda lime containers. V_1 and V_2, valves. *T*, thermometer. *S*, syringe, 40 strokes per minute. *SP*, spirometer. *K*, kymograph and timer.

Fig. 2b. Spirometer. W_1 and W_2, running counter-weights

Fig. 2c. Valve

Fig. 2d. Calibration apparatus

EXPERIMENTS. Data on the O_2 uptake expressed in cubic centimeter per 1000 grams per minute in relation to the age of the rats are presented in table 4 and figure 3. The figures obtained on stock diet are in close agreement with those of Davis and Hastings (17) on rats of corresponding ages, and with those of Griffith and Farris (18). We can also corroborate the statement of Davis and Hastings that the O_2 uptake of males and females does not differ up to the age of 4 months. Nevertheless, we have for other reasons excluded from our tables 4 and 5 results obtained on female rats.

Figure 3 demonstrates that the O_2 uptake per 1000 grams rat per minute between the ages of 15 and 150 days gradually decreases. This result also

TABLE 4

O_2 consumption of male rats in cubic centimeters per 1000 grams per minute according to age

AGE	STOCK DIET (11 ANIMALS)					VITAMIN E DEFICIENT DIET (17 ANIMALS)					VITAMIN E DEFICIENT DIET SUPPLEMENTED BY A SINGLE DOSE OF 1 MGM. TOCOPHEROL ON THE 15TH DAY (11 ANIMALS)				
	No. exper.	Mean O_2 cons.	Maxim. O_2 cons.	Minim. O_2 cons.	SE_m	No. exper.	Mean O_2 cons.	Maxim. O_2 cons.	Minim. O_2 cons.	SE_m	No. exper.	Mean O_2 cons.	Maxim. O_2 cons.	Minim. O_2 cons.	SE_m.
days															
10–15	32	40.1	61	24	1.54	33	43.9	71	27	1.42	30	41.9	52	26	1.13
16–20	34	38.2	57	25	1.19	40	36.6	54	25	1.09	29	35.3	48	26	1.20
21–25	22	37.2	55	29	1.32	46	36.4	48	25	0.80	27	33.6	41	26	0.79
26–30	22	35.6	47	26	1.28	36	38.8	54	25	1.14	20	31.6	44	25	1.09
31–35	10	33.3	41	29	1.21	23	35.0	48	27	1.32	12	28.9	33	24	0.82
36–40	8	31.4	38	25	1.57	15	32.9	39	28	0.89	5	26.0	28	25	0.56
41–50	27	30.8	42	21	0.78	23	26.8	31	17	0.89	12	22.5	28	19	0.82
51–60	11	24.1	29	20	0.86	11	22.8	28	19	0.84	8	21.9	26	19	0.79
61–70	14	24.1	28	20	0.65	17	22.6	26	20	0.49	2	19.5	20	19	0.50
71–80	7	21.7	25	20	0.71	13	20.7	24	19	0.34	5	20.4	22	18	0.82
81–90	10	21.2	25	18	0.85	9	19.8	23	16	0.76	5	18.2	19	16	0.58
91–100	3	20.3	22	19	0.89	20	18.7	25	16	0.57	7	19.4	25	14	1.32
101–120	6	22.2	25	19	0.95	24	19.1	23	16	0.39	5	17.0	19	15	0.55
121–140	3	21.0	25	18	2.10	16	16.8	20	14	0.44	6	16.8	18	14	0.60
141–160						4	20.8	25	18	1.34					

TABLE 5

O_2 consumption of male rats in cc. per 1000 grams per minute according to weight

WEIGHT	STOCK DIET (11 ANIMALS)					VITAMIN E DEFICIENT DIET (17 ANIMALS)					VITAMIN E DEFICIENT DIET SUPPLEMENTED BY A SINGLE DOSE OF 1 MGM. TOCOPHEROL ON THE 15TH DAY (11 ANIMALS)				
	No. exper.	Mean O_2 cons.	Maxim. O_2 cons.	Minim. O_2 cons.	SE_m	No. exper.	Mean O_2 cons.	Maxim. O_2 cons.	Minim. O_2 cons.	SE_m	No. exper.	Mean O_2 cons.	Maxim. O_2 cons.	Minim. O_2 cons.	SE_m
grams															
10–20	24	40.3	58	27	1.90	24	43.7	71	30	1.81	9	42.0	52	26	2.80
21–30	35	40.1	55	25	1.26	56	39.7	54	25	0.92	31	42.5	50	34	0.76
31–40	22	36.1	43	29	0.95	48	37.5	53	25	0.94	21	34.4	41	30	0.75
41–50	19	37.1	48	29	1.25	22	36.9	46	30	0.96	18	33.1	44	26	1.22
51–60	10	31.9	41	26	1.38	15	33.6	46	24	1.28	14	31.0	36	27	0.71
61–70	6	33.2	38	28	1.62	10	33.3	39	28	1.20	7	29.7	33	26	1.03
71–80	3	29.3	34	25	2.07	7	32.0	36	27	1.36	3	27.0	28	26	0.91
81–90	5	33.6	42	29	2.32	6	33.7	39	29	1.50	8	29.3	33	25	1.08
91–100	7	30.9	34	27	0.94	4	29.8	31	28	0.63	4	29.2	33	25	1.75
101–120	14	29.6	33	25	0.93	9	26.9	29	25	0.45	7	27.7	29	26	0.54
121–140	10	29.4	39	25	1.58	15	25.8	31	19	0.89	5	25.0	27	22	1.09
141–160	8	25.0	29	21	0.93	7	25.0	29	22	1.19	8	22.1	28	19	1.30
161–180	6	23.7	28	20	1.23	8	23.1	28	17	1.21	1	20.0	20	20	—
181–200	17	23.6	28	20	0.52	8	20.5	24	16	1.16	6	21.8	24	20	0.63
201–225	8	21.8	25	18	0.88	14	20.9	24	17	0.43	4	21.8	22	21	0.33
226–250	9	20.1	22	18	0.54	19	19.6	24	15	0.50	4	19.7	21	19	0.65
251–275	3	21.0	24	19	1.26	21	18.8	23	16	0.43	8	19.5	25	16	0.98
276–300						22	19.0	25	14	0.51	3	17.8	19	16	0.91
301–325						11	18.9	25	16	0.78	3	16.3	18	14	1.21
326–350						5	16.8	19	15	0.66	4	16.8	19	14	1.30
351–375											6	17.3	18	17	0.22

corresponds to that obtained by Benedict and Macleod and by Davis and Hastings. The average values for the vitamin E deficient animals do not differ greatly from those of the ones on a stock diet, except for a dip in the curve between the ages of 15 to 30 days, the significance of which will be discussed later.

The vitamin E deficient animals whose diet was supplemented by a single dose of 1 mgm. tocopherol on the 15th day after birth showed a lower O_2 uptake from the 15th day to the 70th day. The statistical evaluation[7] indicated that the difference between the two groups became significant after the 20th day of life, and remained so until the 70th day except for the period of 51 to 60 days. *The single protective dose of 1 mgm. of alpha-tocopherol was therefore sufficient to change the O_2 consumption of the E deficient rats from weaning until sexual maturity.*

One might object that the weight of the animals was not taken into consideration. This objection seems justified inasmuch as the "protected" animals were usually heavier than unprotected ones, an observation that will be discussed in a later publication. We have therefore computed the O_2 consumption in cubic centimeters per 1000 grams per minute, according to the weight of the animals (table 5). The curve obtained by this presentation is similar to that in figure 3. The O_2 consumption again shows a gradual decrease; the curves of the stock rats and of the vitamin E deficient ones are similar; the values of the vitamin E deficient animals are higher than those of the protected ones over a range of from 30 to about 100 grams. The statistical evaluation again indicates a significant difference between individual points from 30 to 90 grams. The differences of the means are, however, slightly less pronounced than those in table 4 in which the O_2 uptake was computed according to the age.

Despite the overlapping of individual results, this also indicates that the single dose of alpha-tocopherol was sufficient to decrease the O_2 uptake of the rats from the time of weaning until sexual maturity.

The results have also been computed in relation to the surface area, which was calculated by Lee's formula, $S = 12.44\ W^{3/5}$, where S stands for the surface area in centimeters^2 and W for the weight in grams (19). The O_2 consumption in all three groups increased up to a body weight of about 100 grams and became constant thereafter. The initial increase is not due to the inapplicability of

[7] The data were statistically analyzed by finding the standard deviation, $\sigma = \sqrt{\frac{\Sigma^2}{n-1}}$ where x repreents the difference from the mean and n the number of experiments. The standard error of the mean, SE_m, was calculated by the formula, $SE = \frac{\sigma}{\sqrt{n}}$, and the standard error of the difference of the means by the formula, $SE_{m_1 - m_2} = \sqrt{SE_{m_1}^2 + SE_{m_2}^2}$. If the difference of the means divided by SE_m showed a factor > 2, a significant difference could be assumed. The significance was even greater if subsequent points in the curve showed factors > 2.

For the help granted to us in the statistical analysis of our results, we are indebted to Prof. John W. Fertig of the De Lamar Institute of Public Health, Columbia University.

Lee's formula to small animals, since it has been shown by him to apply to animals of all weights. Again one finds that the curves of the vitamin E deficient and the stock rats are similar, whereas that of the "protected" animals is lower over the range of 30 to 90 grams; the statistical evaluation again confirms the significance of the differences.

An interesting feature of the O_2 consumption is observed during the period of the development and subsequent spontaneous regression of the muscle lesions. The animals usually develop general signs between the 18th and 22nd day and the survivors recover within 3 to 10 days after the onset. We observed signs of muscular disease in 13 of the 19 unprotected animals. In 8 of the 13 rats with signs, and in 2 of the 6 rats without signs,[8] we noted a considerable decrease of the O_2 consumption during the development of the disease (fig. 4). The low value disappeared when recovery began. In 3 cases in which the experiment was terminated by the death of the animal, the O_2 consumption showed a continual decline. Among 14 protected animals a similar curve was seen only once.

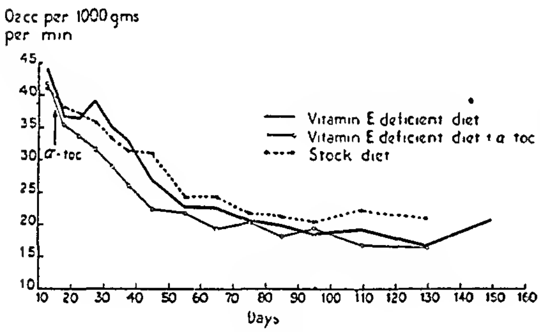

Fig. 3

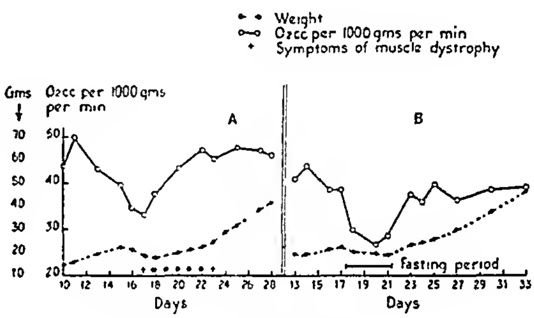

Fig. 4

Fig. 3. Average O_2 consumption of rats on experimental diets at different ages.

Fig. 4 A. Depression of O_2 consumption in a vitamin E deficient rat during dystrophic period. B. Depression of O_2 consumption produced by fasting in a normal rat.

In order to find out whether or not the described effect was specific for the vitamin E deficiency, or whether it was a consequence of the reduced nutrition and activity of the animals during the acute phase of the disease, comparable determinations were made on rats which were partially starved from the 18th to the 21st day after birth. They were the offspring of mothers maintained on a stock diet. We chose that time because it is the period during which the vitamin E deficient animals showed the dip in the curve. The animals were kept without food and water, but each day they were returned to their mother for two hours because it seemed probable that the vitamin E deficient animals secured at least some nourishment despite the disease. Figure 4 B shows the results obtained in an individual animal. The curve is very much like the ones obtained in the majority of the vitamin E deficient rats. The results of the tests cor-

[8] The occurrence of severe muscle lesions in the absence of clinical signs has been recorded by Pappenheimer (J. Mt. Sinai Hosp. 7: 65, 1940) and by Telford, Emerson and Evans (Proc. Soc. Exper. Biol. and Med. 45: 135, 1940).

respond with the experiments of Benedict and Macleod, who found that 24 hours' starvation reduces the O_2 uptake of young rats by 28 per cent. We performed the experiment on 7 rats from three different litters. The fasted rats were compared with their normal litter-mates. In the 7 fasted rats, the dip in the curve was observed 6 times; among the normal litter-mates it did not appear at all. These control tests indicate that the reduced O_2 uptake of the vitamin E deficient rats is a consequence of the starvation during the period of muscular dystrophy, rather than a specific effect of the vitamin deficiency.

In order to see whether the difference between the "protected" and the "unprotected" animals in the later stages of the experiment is a protracted effect of the starvation during the lactation period, the O_2 consumption of the partially starved animals on stock diet was compared with that of their unstarved litter-mates. One finds that there is a dip in the curve during the period of starvation, but later the curves up to 85 days are identical. The difference in O_2 consumption between the vitamin E deficient group and their protected litter-mates is therefore not due to transient inanition during the acute disease period.

DISCUSSION. These determinations show that the total O_2 consumption of young rats on a vitamin E deficient diet, which have received a single dose of alpha-tocopherol on the 15th day, is lower than that of untreated litter-mates. The difference persists from the time of weaning until the onset of sexual maturity.

The living animal thus reacts in a fashion comparable to that of excised muscle *in vitro* and the question may be here discussed as to whether the increase in total O_2 consumption may be attributed to this change in the metabolism of the skeletal muscle. The average percentage increase based on weight in the living animal during the significant period is about 11.4 per cent, whereas that in the excised muscles was 30 per cent. The weight of the total skeletal muscular tissue is found by dissection to be about one-third of the body weight. If the increase of the O_2 consumption were wholly referable to the muscle tissue, and if the respiration of the muscle tissue *in vitro* were of the same order as in the living animal, one would expect an increase of roughly 10 per cent. Considering the many assumptions involved in such calculation, the order of magnitude of the change is not beyond what might be anticipated. That this increase in metabolism does not affect all the viscera is indicated by the fact that the *in vitro* O_2 consumption of the liver is unaltered in E deficient rats.

One may also raise the question as to a possible influence of the thyroid in stimulating the metabolism. Evidence gathered from the literature is conflicting. Singer (20) and Barrie (21) found hypoplastic glands in vitamin E deficient rats. Telford, Emerson and Evans (8) were unable to confirm this. Morgulis and Spencer (22) found no change in the relation of dry weight of the thyroid gland to body weight in dystrophic rabbits. On the other hand, Biddulph and Meyer (9) found a two-fold increase in the weight of the thyroid of male rats on vitamin E deficient diet. The addition of iodine to their vitamin E deficient diet reduced the thyroid weight and the increased metabolism to

normal, and the hypertrophy is ascribed to the low iodine content of their diet and drinking water.

In our experiments, a low iodine intake as a possible cause of thyroid hyperfunction can be positively excluded. On the basis of an average daily food consumption of 10 grams, our rats ingested 0.77 mgm. of iodine, calculated on the KI content of the Hawk-Oser salt mixture used in the experimental diet. We noted no difference in thyroid structure or weight in 3 unprotected and 2 protected rats kept for 5 months on vitamin E deficient diet.

A disturbing feature of our observations is that the stock rats showed an O_2 consumption which was much nearer to that of the vitamin E deficient than to that of the "protected" rats. We made a similar observation in our study of the metabolism of excised muscles. There also it was found that the average values in the E deficient animals matched those of the stock rats; the muscles of "protected" rats yielded considerably lower figures. Taken at their face value, the data would lead to the conclusion that the O_2 consumption of the vitamin E deficient rats is normal, that of the tocopherol treated rats, depressed. But wide difference in the composition of stock and experimental diets raises the question as to whether there may not have been other dietary factors than the vitamin E content which influenced the total metabolism. The possiblity that the stock diet used is low in its vitamin E content seems remote, since it maintains fertility in the breeding colony, prevents testicular atrophy and late muscular dystrophy, and contains 3.2 mgm. per cent of alpha-tocopherol by bio-assay.

SUMMARY

1. The *in vitro* consumption of oxygen by the skeletal muscle of young rats born of vitamin E deficient mothers was found to be higher than that of litter-mates receiving a protective dose of 1 mgm. synthetic alpha-tocopherol acetate on the 15th day.

2. This difference may be noted in the absence of microscopic alterations in the muscle fibers.

3. The skeletal muscle of chicks on vitamin E deficient diet had a higher *in vitro* consumption of O_2 than that of chicks on a stock diet or on an E deficient diet supplemented by dl-alpha-tocopherol acetate.

4. No effect of vitamin E deficiency upon the *in vitro* uptake of rat liver could be detected.

5. The total O_2 consumption of rats on a stock diet, on a vitamin E deficient diet, and on a vitamin E deficient diet supplemented by a single dose of synthetic dl-alpha-tocopherol was determined over a period ranging from 11 days to 5 months of age.

6. Following a single dose of alpha-tocopherol given on the 15th day, the O_2 consumption was significantly lower than that of the untreated litter-mates. This difference persisted from the time of weaning (or recovery from the muscular dystrophy) until the 60th to the 70th day, corresponding to a weight range of about 30 to 100 grams.

7. During the development of the muscle disease (from about the 18th to 22nd day after birth), the O_2 consumption of the E deficient group was decreased; this effect seemed to be a consequence of the reduced nutrition and activity during that period, since it could be imitated in partially fasted rats of the same age. Partial starvation towards the end of the nursing period did not, however, lead to protracted changes in the O_2 consumption of rats on a stock diet.

8. The O_2 consumption of rats on a stock diet was found to be similar to that of the vitamin E deficient ones. No satisfactory explanation for this fact can be given.

We wish gratefully to acknowledge the skilled collaboration of Dr. Constantino Mignone, Fellow of the Rockefeller Foundation, in the first part of this work; and to thank Mrs. Thelma Stout and Mrs. Claudia Schogoleff for technical assistance.

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THE EFFECT OF HISTAMINE ANTAGONISTS ON GASTRIC SECRETION

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Experimental studies concerning the effect of the histamine antagonist, thymoxyethyldiethylamine (929F), on gastric secretion induced by histamine (1) have been extended to determine the effect of 929F on gastric secretion induced by pilocarpine. In addition, the histamine antagonist (2, 3), N-phenyl-N-ethyl, N′-diethyl, ethylenediamine (1571F), was employed in experiments designed to ascertain whether the gastric secretory response to histamine could be suppressed or wholly eliminated. Pilocarpine and food were also used as secretory stimulants in an attempt to determine whether 1571F[2] acted specifically as a histamine antagonist. Any drug having the ability to specifically antagonize the powerful stimulating action of histamine on the gastric secretory glands could be advantageously used to investigate the relationship, if any, of histamine to gastric secretion following the ingestion of food or under other conditions.

Methods. Heidenhain pouch dogs trained to stand quietly in stocks were used. The animals were fasted for at least 20 hours before the experiments were begun.

All drugs were freshly prepared (5.0 per cent solution) and injected subcutaneously. 929F and 1571F were given in doses of 10 mgm./kgm. In the studies with 1571F and histamine, the double histamine test was employed as previously described (1). The maximal non-toxic dose for our dogs, 10 mgm./kgm., was injected 30 minutes before the second histamine injection.

Rectal temperatures were determined at 20 or 30 minute intervals before and for 3 hours following the administration of the drugs (929F or 1571F). This was considered necessary because in preliminary experiments we had found that 1571F in subcutaneous doses of 20 or 40 mgm./kgm. produced muscular tremors, convulsions and pyrexia. However, no significant temperature rises were noted in dogs treated with 10 mgm./kgm. of 1571F or 929F.

In the pilocarpine experiments a dose of 2.0 mgm. of pilocarpine hydrochloride was injected 30 minutes after 929F or 1571F. This dosage of pilocarpine was selected because we found that it induced a volume of gastric juice approximately equal to that obtained following the injection of 0.5 mgm. of histamine diphosphate, the dosage employed in the histamine experiments.

In the food experiments the stimulus consisted of a meal of 200 cc. of milk and 200 grams of Kennel Ration. Since the gastric secretory response to this

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[2] The drugs 929F and 1571F were supplied by Parke, Davis & Co.

stimulus continues for 1 to 6 hours, a dose of 10 mgm./kgm. of 1571F was given one and three hours after the meal stimulus. Corresponding experiments with 929F were not conducted because it had been shown by Loew and Chickering (1), that this drug did not inhibit the histamine-induced gastric secretory response.

The gastric juice was collected and the volume recorded at 20 to 30 minute intervals. Each sample was then titrated *in toto* for free and total acid, using Töpfer's reagent and phenolphthalein, respectively, as indicators.

RESULTS. A. *1571F and double histamine.* In 15 experiments on 3 dogs injections of 1571F 30 minutes before the second histamine stimulus reduced the volume, total acidity and free acidity of the gastric juice, 21.9 per cent, 35.2 per cent, and 47.2 per cent respectively (table 1). These percentages were obtained by comparing the output after 1571F and the second histamine stimulus with control data consisting of the output after the first histamine injection.

TABLE 1

Gastric secretion following histamine stimulation in dogs pretreated with 1571F

	NUMBER OF EXPERIMENTS	NUMBER OF DOGS	VOLUME	TOTAL ACID	FREE ACID
			cc.	*mgm. HCl*	*mgm. HCl*
Control					
Histamine	15	3	15.5	52.2	46.4
Treated					
1571F and histamine	15	3	12.1	33.8	24.5
Variation from control (%)			−21.9	−35.2	−47.2
Control					
Histamine #1	15	3	16.4	52.9	46.9
Control					
Histamine #2	15	3	15.5	51.0	44.9
Variation of histamine #2 from histamine #1			−5.4	−3.5	−4.2

When pretreatment with 1571F was omitted the outputs of gastric juice following the first and second histamine injections were practically the same in volume, free acidity and total acidity (table 1). We have also noted that in a given dog under nearly identical conditions, the secretory output was fairly constant following the first and second histamine injections on the same and different days.

B. *1571F and pilocarpine.* In 15 experiments on 3 dogs the administration of 1571F 30 minutes before pilocarpine stimulation reduced the volume, total acidity and free acidity of the gastric juice, 13.5 per cent, 36.8 per cent and 47.2 per cent, respectively (table 2). This degree of inhibition is nearly identical to that which obtained when histamine was used in animals pretreated with 1571F.

C. *1571F and food.* In 10 experiments on 4 dogs administration of 1571F at one and three hours after the meal stimulus failed to modify the gastric secretion as compared with control experiments with food alone. The differ-

ence in mean values for volume, total acidity and free acidity did not exceed 5.0 per cent (table 2).

D. *929F and pilocarpine.* In 12 experiments on 3 dogs injections of 929F 30 minutes before pilocarpine failed significantly to depress the secretory output. The variations from control experiments were 12.0 per cent, 3.8 per cent, and 14.5 per cent for volume, total acidity and free acidity respectively (table 2).

DISCUSSION. The degree of inhibition of gastric secretion produced by injections of 1571F in dogs stimulated with histamine or pilocarpine was nearly identical. In each instance, computation of the probable error of the mean differences ($M_1 - M_2 \pm$ P.E.) revealed that the data were statistically significant. No unequivocal evidence has been presented to indicate that pilocarpine exerts its stimulating effect on gastric secretion through the mediation

TABLE 2

Gastric secretion following pilocarpine stimulation or a meal stimulus in dogs treated with 929F or 1571F

	NUMBER OF EXPERIMENTS	NUMBER OF DOGS	VOLUME	TOTAL ACID	FREE ACID
			cc.	*mgm. HCl*	*mgm. HCl*
Control					
Pilocarpine	15	3	14.0	32.0	26.9
Treated					
1571F and pilocarpine	15	3	12.1	20.2	14.2
Variation from control (%)			−13.5	−36.8	−47.2
Control					
Pilocarpine	12	3	12.0	36 1	30 3
Treated					
929F and pilocarpine	12	3	13 4	34 7	25 9
Variation from control (%)			+12.0	−3.8	−14.5
Control					
Meal	10	4	91.8	419.8	387.1
Treated					
Meal and 2 doses 1571F	10	4	89 6	399.0	378.1
Variation from control (%)			−2 3	−4 9	−2 3

of histamine. Therefore our data do not suggest that 1571F specifically antagonizes the stimulating effect of histamine on the gastric secretory cells. It is possible, however, that 1571F antagonized the pharmacological activity referable to distinct chemical configurations present in both histamine and pilocarpine.

Even though 1571F reduced the secretory output following histamine and pilocarpine injections, it failed to inhibit the secretion induced by a meal stimulus. Hence it may be concluded that 1571F did not influence the humoral or hormonal factors related to gastric secretion which operate following a natural stimulus such as the intake of food.

Some investigators have presented evidence which suggests that the hormone, gastrin, may be identical with histamine. However, in relation to this question

it is interesting to note that in our experiments 1571F antagonized injected histamine but failed to antagonize the humoral and hormonal factors which elicit gastric secretion following a meal stimulus.

The inhibition of gastric secretion by 1571F in animals stimulated with either histamine or pilocarpine can not be referable to concomitant pyrexia. At no time during the periods following the administration of 1571F did the average rectal temperatures exceed by more than 0.3°F. the average control temperatures taken just before treatment with 1571F or during the histamine and pilocarpine control experiments. No depression of gastric secretion occurred in the food experiments where two doses of 1571F would be expected to increase pyrexic effects.

Since 929F potentiated the stimulating action of histamine on the gastric secretory cells (1), it was interesting to note that the drug failed to potentiate the action of pilocarpine.

Burchell and Varco (5) have recently reported their inability to demonstrate that the two histamine antagonists, 929F and 1571F, diminish the secretory response of gastric pouches (Heidenhain) following histamine stimulation. The dose of histamine which they employed was several times greater than the minimal amount which we found adequate to induce a moderate gastric secretory response in our animals. Our control data and the data reported by others (6) firmly establish the reliability of the double-histamine assay procedure when proper precautions are taken regarding the number of experiments conducted, the health of the animals, and the use of non-toxic doses of drugs or biological preparations. Hyperpyrexia referable to parenteral administration of substances which contain pyrogens, or to muscular tremors or convulsions coincident with drug action will reduce gastric secretion.

It was not possible to employ larger doses of 1571F or 929F in an attempt to determine whether these drugs antagonize the effect of histamine on the gastric cells. The former drug, in doses of 20 and 40 mgm./kgm., subcutaneously, caused normal dogs to become apprehensive, nervous, excitable and, finally, produced muscular tremors, tonic convulsions, and hyperpyrexia. The reactions were noted 15 to 45 minutes after administration of the drug. It was previously mentioned (1) that 929F induces retching, vomiting, and defecation when quantities of 20 or 30 mgm./kgm. were administered subcutaneously. Others (2–5) have commented on the symptoms and untoward reactions observed following administration of 929F and 1571F.

CONCLUSIONS

1. The data do not support the belief that 1571F is a specific antagonist to histamine. Although the drug reduced the gastric secretion in dogs with gastric pouches (Heidenhain) following histamine stimulation, the reduction in secretion was nearly identical after pilocarpine stimulation. 1571F did not modify the gastric secretion of gastric pouch dogs stimulated with food.

2. Pretreatment with 929F failed significantly to modify the secretion from gastric pouches in dogs injected with pilocarpine.

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THE DEVELOPMENT OF RESISTANCE BY RATS AND GUINEA PIGS TO AMOUNTS OF TRAUMA USUALLY FATAL[1]

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A method for the experimental production of graded amounts of trauma has been previously studied in this laboratory and described by Noble and Collip (1). It was found that animals which died following trauma showed many of the characteristic findings ascribed to the condition of "shock". The observations presented supported the hypothesis that the production of a toxic substance in the tissues was the etiological factor in traumatic shock. The present paper reports a continuation of research based on the toxin theory of shock. Rats and guinea pigs have been subjected to trauma by the previously described method. Since this procedure did not require the animal to be anesthetized and caused only a minimal degree of hemorrhage, it was especially suited for a study of the effects of repeated trauma. In the initial experiments it was found that rats subjected to small amounts of trauma rapidly acquired a resistance so that they could withstand a degree of trauma otherwise fatal. A detailed study of this phenomenon forms the basis for this communication.

Methods. Since the procedure used to produce trauma has been previously described in detail (1), only a brief résumé is included. The animal, after taping of the paws, is placed in a circular drum which has two projections on the side. During rotation of the drum at a speed of 42 r.p.m. the animal is alternately carried a short distance up the side on the projection and then dropped. The amount of trauma, therefore, is directly related to the number of turns of the drum, and is reflected on the mortality curve. The animals used in these experiments have been maintained under the same conditions as those previously described, so that the results would be comparable.

To make rats resistant it has been customary to use an animal of 120 to 140 grams and subject it initially to 200–300 turns, since this seldom causes mortality. This is repeated after 2 days, and at intervals of 2 or 3 days the number of turns is increased to 400, 600 and 800. Over a period of from 12 to 14 days the animals are raised from 200 to 1000 turns. As a standard procedure they are subjected to 1000 turns every week thereafter until used for other experiments. With guinea pigs the same principle is followed, except that due to their increased susceptibility only 125 turns are given initially, and the number is increased by 25 to 50 over a slightly longer period than for rats. The speed with which the number of turns may be increased can be judged by following the weight curves of the animals. With correct spacing the animals do not lose in weight after the initial run. For adrenalectomized animals the number of initial

[1] The findings recorded in this paper are a continuation of those contained in reports submitted to the National Research Council of Canada, dating from 30th July, 1941.

turns is reduced, and then raised more slowly, due to their increased susceptibility.

Results. *Production of resistance in rats.* When an untreated rat is subjected to 1000 turns initially it has been found in a large series of experiments that only very exceptionally does an animal survive. This appears to be true in rats weighing from 120 to 300 grams. Although large rats are slightly more resistant than small ones, the amount of trauma following 1000 turns is of sufficient magnitude to cause 98 to 100 per cent mortality. Rats which are able to tolerate 1000 turns successfully are referred to for the purposes of this paper as resistant animals. At present, out of 106 male rats 105 have been made resistant, and have withstood 1000 turns on at least 3 occasions. During the development of resistance only one rat has died, and that was following the initial run. At present, therefore, 105 animals have been subjected to an amount of trauma almost invariably fatal to an untreated rat, without mortality. Actually these 105 rats have received 1000 turns a total of 919 times. In these experiments the amount of trauma was increased gradually, as indicated. In most cases the time intervals and amount of trauma were judged by the weight curves of the animals. After the initial run some loss in weight usually occurred over 24 hours. Following this, however, the rats gained weight slowly despite subsequent trauma. If a loss in weight continued or was marked, the number of turns was lowered and a longer rest period given between runs. In general, however, the animals appeared to develop a resistance with unexpected rapidity. Once the rats were made resistant they have received 1000 turns weekly or twice weekly. In most cases this has continued for 5 or 6 weeks before the animals were used for other experiments. In one case 8 rats have received 1000 turns on 26 occasions over the past 6½ months, and at present show no indication of ill health or loss of resistance.

Appearance of resistant rats. Following the first few runs the rats appeared much less affected by the trauma than would be expected from observations on control animals. When a resistant rat was subjected to 1000 turns its general appearance differed markedly from that of an animal subjected initially to severe trauma. A few moments after removal from the drum the resistant rat appears quite normal, runs about the cage, exhibits a normal response to outside stimuli, and never shows the prostration or collapse described as typical of this form of trauma. Such animals do not drink excessively and there is no initial phase of anuria; in some cases mild polyuria may be found. Subsequently, they do not develop diarrhea.

If a resistant rat is examined after receiving 1000 turns the appearance is in sharp contrast to an animal killed by trauma. The heart appears to continue to beat strongly and is not congested or dilated; the cut tissues bleed readily—suggesting a normal blood pressure; very little bruising or hemorrhage can be found even over the bony prominences. The abdominal organs and gastro-intestinal tract appear essentially normal and show no sign of the congested condition seen in unresistant rats. The lungs and kidneys are not affected. Congestion of the brain is absent and sub-arachnoid hemorrhage is not seen.

The adrenals appear to be within normal limits. If an animal which has only recently been made resistant to 1000 turns is killed, it will not, however, show such a normal picture as outlined above. In this case some of the changes previously reported for normal rats may be found, although they are of much less intensity. As the degree of resistance of the animal increases the abnormal findings become progressively less.

Blood findings in resistant rats. As the general appearance of the resistant animal after trauma would suggest, little evidence of concentration of the blood has been found. The usual hemoconcentration associated with this form of trauma in the normal rat was not encountered in the resistant animal. Changes in hemoglobin values have been very small when followed for individual rats and have indicated that, if anything, hemodilution to a mild degree may follow trauma. In addition, it has been noted that the hemoglobin values of resistant rats tend, as an average, to be slightly lower than those of normal rats, although marked variations occur.

It has been noted that normal rats which die immediately following trauma develop rigor mortis after a few minutes. However, when resistant rats were killed following trauma this immediate rigor mortis did not take place (although it sets in later, as is found when a control untraumatized rat is killed). Lactic acid determinations on the blood and muscle of some of these animals were kindly carried out by Dr. A.H. Neufeld, of this Institute. In the normal rat after trauma very high figures were found; the blood increased from a normal of 20 to 30 mgm. per 100 cc. to 100 to 200 mgm. or even higher. In the case of 7 resistant rats subjected to 1000 turns the blood lactate was much lower and ranged from 26 to 53, an average of 40.4 mgm. per 100 cc. Other studies have failed to demonstrate that the resistant rat can handle lactic acid more readily than the normal.

Factors affecting resistance. Although for the purposes of this paper rats are considered resistant when they survive 1000 turns, it is possible to continue to increase this considerably. In one case 4 rats were rapidly raised to 1000 turns in 12 days. Then at 5 to 6 day intervals the number was increased to 1500, 1800, 2000, 2500 and 3000. One rat died after 1500, two after 2500, and one after 3000. It is believed that if the increase in trauma had been made more gradual, that even greater resistance would have developed. After such prolonged periods of trauma some of the rats may have difficulty in eating, due to broken teeth, etc., and special care is required to maintain their general condition.

The amount of trauma necessary for the development of detectable resistance has been investigated by a different type of experimental procedure. In this case 12 rats received 300 turns on two successive days. At 3, 8 and 15 days after the initial run each group of 4 received 1000 turns. In the first two cases only evidence that some resistance had developed was indicated by a lowered percentage mortality, or as an increased survival time before death (2 to 48 hrs.). This form of experiment would appear to be advantageous where a long survival time is desired, since with normal animals death occurs so rapidly after severe trauma that they are unsatisfactory for certain types of tests.

A number of rats have been set aside to determine the length of time the resistance continues after the last exposure to trauma. The rats were made resistant in the usual way and received 1000 revolutions on at least 3 occasions. After an interval of one month 9 of 10 animals retained their resistance to 1000 turns. After 3, 4 and 5 months 2 of 3 rats survived in each case. The resistance, when once established, therefore, persisted for a considerable period of time. During an epidemic of gastro-enteritis which developed in the rat colony it was noted that 4 resistant rats had apparently contracted the disease. While still in relatively good health, the animals received 1000 turns, and in each case death followed.

Resistance after anesthesia. Despite the fact that the legs of the animals were taped together, it was suggested that the resistance might be related to the rat learning how to fall and minimize the degree of trauma. To rule out this factor, 3 resistant rats were anesthetized with nembutal and then given 1000 turns. All these animals survived, although co-ordinate movements were abolished and muscular relaxation was complete. There was no evidence of rupture of the spleen, as frequently occurred in normal rats traumatized while under nembutal anesthesia.

Resistance in adrenalectomised rats. Two sets of experiments were devised to determine whether adrenalectomised rats could be made resistant. In the first group the rats were maintained by drinking 0.9 per cent NaCl instead of water, and the second group in addition received pellets (80–90 mgm.) of desoxycorticosterone acetate implanted subcutaneously. Animals treated in such a manner are, as previously reported, very sensitive to trauma, so that after 500 turns a mortality of 75 to 85 per cent is found. In order to make these animals resistant, they received 200 turns initially, and the amount of trauma was increased by 100 turns over 2 to 3 weeks. Of the first group of 8 rats, three died early in the experiment after 400 turns, five tolerated 600 turns successfully, and one rat survived after 900. This rat was then given water to drink instead of saline and died in 4 days. In the second group of 6 rats all tolerated 600 turns, and four 700 turns.

Resistance in guinea pigs. It has been found that guinea pigs readily may be made resistant by a method similar to that described for rats. Since this species shows almost 100 per cent mortality after 300 turns, it is necessary to commence with only 125 turns. After 4 to 5 day intervals the number of turns was increased 25 at a time. Starting with 16 animals, it was possible to have 13 of them survive after 400, and 3 animals were taken as high as 600 turns. During this process some animals were subjected to trauma as often as 26 times.

Discussion. The results which have been obtained show that by gradually increasing the amount of trauma, rats and guinea pigs eventually become resistant to normally fatal doses. Since it is believed that the drum method of applying trauma results in a condition of shock, it would appear that animals may be made resistant to this condition. The normal and the resistant animal show a striking difference in appearance after exposure to trauma. Whereas the normal animal is obviously affected and dies with severe diverse pathological

changes, the resistant rat appears normal in every respect. Concentration of the blood does not occur in the resistant rat. It has been possible to expose 105 rats, without mortality, to degrees of trauma almost inevitably fatal to normal rats. The type of resistance which develops appears to have some curious features. The onset is rapid, and in a very short time the animal can withstand large amounts of trauma. Once the resistant state is established it has not shown any tendency to diminish even though rats have received trauma over periods of $6\frac{1}{2}$ months. However, 4 rats which contracted gastro-enteritis apparently lost their resistance to trauma. The resistant condition may persist for several months after the last exposure to trauma. Since the actual mechanism of death in the animals exposed to this type of trauma has not yet been clearly defined, it is difficult to postulate what type of resistance develops. Such factors as "learning" have been ruled out by the demonstration that even when animals were completely anesthetised they maintained their resistance. Since the preliminary research on this type of trauma was instituted, the view was held that the results could best be explained by the theory of a liberation of a toxic substance by damaged tissue. It is possible, if this theory is correct, that the resistant animals become immunized by the production of some antitoxin. In this connection experiments designed to transfer protection by the blood of resistant animals have so far proved unsuccessful. However, at present it is thought that some such detoxifying mechanism is involved and that further work may clarify the question of whether active or passive immunization may be possible. Whether rats resistant to trauma inflicted by the drum method are resistant to other forms of trauma has not yet been established.

Using the drum method for producing trauma it has been difficult to assess to what degree direct mechanical damage might be related to the death of the animal. Some concussion of the brain and nervous system must result from the nature of the trauma, and it has been suggested that intracranial hemorrhage may be a factor in producing mortality. Whether animals showing evidence of brain hemorrhage should be discarded from the results is difficult to assess. In the case of rats in the present work, this has not been considered as a separate factor. When resistant rats are subjected to even extreme degrees of trauma, they do not die, and postmortem examination has not revealed any brain hemorrhage. In this case one would expect any type of mechanical injury to be more prominent than in the case of normal animals. It is possible, however, that with the resistant state the results of mechanical damage are minimized in some manner. In a previous paper (2) it was reported that treatment with adrenal cortical extracts, while restoring the resistance of adrenalectomized rats, would only slightly raise the resistance of normal rats to trauma. The observations in this paper would indicate that the adrenal glands are not essential for the development of resistance to trauma. In the case of adrenalectomized rats, as in normals, it has been possible to make them resistant to usually fatal degrees of trauma. Whereas the adrenals undoubtedly play some rôle in the general maintenance of the animal's normal tolerance to trauma, they are not essential for the development of the special type of resistance described.

Up to the present time the procedure described is the only treatment after which rats have consistently survived 1000 turns. In a recent report (3) it was found that ascorbic acid prevented mortality following a certain type of trauma. Using the drum method as described, pretreatment with ascorbic acid in doses of 300 mgm. per kgm., either by single or repeated subcutaneous injection, or by mouth, has not prevented mortality after 1000 turns.

From the findings reported it has been possible to establish a colony of resistant rats and use them for comparative studies with normal and traumatized rats. It is hoped that with such animals for assay purposes the investigations for a specific toxic substance in tissues may be expedited.

SUMMARY

By gradually increasing the degree of trauma, as applied by the drum method, it has been possible to make rats and guinea pigs resistant to amounts of trauma fatal to normal animals.

Resistant animals, when exposed to severe trauma, appear normal in every respect and do not show any of the signs of shock as encountered in normal animals.

The resistant condition persists after numerous repetitions of trauma, is not affected by nembutal anesthesia, and is still present some months after the last exposure to trauma.

Adrenalectomized rats have been found to develop a resistant state to trauma similar to intact animals.

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EFFECT OF THE TOTAL LOSS OF PANCREATIC JUICE ON THE BLOOD AND LIVER LIPIDS[1]

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The status of lipocaic as an internal secretion of the pancreas depends in part upon recognition of the fact that the depancreatized animal is not returned to a normal state by the adequate administration of insulin. There is nearly general agreement at the present time that the depancreatized dog will not survive for long if treated only with insulin and that at death a profound infiltration of fat in the liver is usually found. Animals maintained on high fat diets may develop severe fatty livers and die within six weeks, whereas those fed a low fat or fat-free diet may survive for many months (1). The oral administration of fresh pancreas corrects the deficiency and when given along with insulin permits the depancreatized dog to survive indefinitely in a good nutritive state. The conclusion from this laboratory (2) that the beneficial effect of pancreas in this connection cannot be accounted for on the basis of its content of lecithin or choline has been accepted by Best (3) and McHenry (4), both of whom have contributed so much to our understanding of the lipotrophic action of these substances. That pancreatic enzymes are not necessary for this effect is indicated by the fact that pure choline is active and also by the fact that preparations of lipocaic free from pancreatic enzymes have been secured (1). There remains the possibility that the specific substance in pancreas, lipocaic, is excreted in the pancreatic juice and normally reabsorbed from the intestine. In this event the total deviation of the pancreatic secretion to the exterior by means of an appropriate fistula might be expected to cause fatty infiltration of the liver and the other sequelae of lipocaic deprivation.

Methods. The total external loss of pancreatic juice was accomplished by a minor modification of the method previously described from this laboratory (5). Adult female dogs were operated upon under morphine-ether anesthesia and with the usual aseptic precautions. The pancreas was separated from the first portion of the duodenum to a point 2 cm. below the entrance of the common bile duct. Several small pancreatic ducts were usually divided and ligated in this process. The duodenum was then transected immediately below the bile duct and again 6 cm. lower down. The short segment of duodenum thus isolated contained the lower major pancreatic duct. A gold plated cannula was placed in this isolated segment, the ends closed, and the whole carefully wrapped in

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omentum. The cannula was led to the outside through a small stab wound. The remaining proximal and distal ends of the duodenum were anastomosed by end to end suture. The juice was collected continuously throughout the life of the animal by means of sealed rubber bags attached to the cannula. The amount secreted ranged from 500 to 2000 cc. daily and necessitated the intravenous administration of large amounts of Ringer's solution to prevent acidosis and dehydration. Calcium carbonate and powdered bone meal were given by

TABLE 1

The effect of complete loss of pancreatic juice on the blood lipids

DOG NO.	WEEKS AFTER PRODUCTION OF PANCREATIC FISTULA	TOTAL BLOOD LIPIDS	DOG. NO.	WEEKS AFTER PRODUCTION OF PANCREATIC FISTULA	TOTAL BLOOD LIPIDS
		mgm. per cent			*mgm. per cent*
11	Control	710	16	Control	640
	2	742		2	587
	3	601		3	727
	4	760		4	620
	5	596		5	533
12	Control	608		6	540
	2	722		7	607
	3	928		8	505
	4	917	17	Control	644
13	Control	670		1	663
	1	640		2	550
	3	688		3	773
	4	680		4	580
	5	660		5	833
14	Control	457		6	613
	2	560		7	777
	4	602		8	540
15	Control	666		9	533
	4	629		10	410
				11	465
				12	297
				13	370
				14	537
				16	587
				17	513
				18	702
				19	670

mouth to prevent the development of gastric and duodenal ulcers. The diet was that commonly given to depancreatized dogs in this laboratory and consisted of white bread, whole milk, meat and suet, supplemented with cod liver oil and brewer's yeast. The fat content of this diet is approximately 40 per cent.

The blood lipids were determined by a modification of Bloor's method on weekly samples taken from certain of the animals as indicated in table 1. An autopsy was performed at the conclusion of the experiment to determine the

condition of the liver and pancreas and to make certain that no ducts remained whereby pancreatic juice could enter the duodenum. The amount of fat in the liver was estimated by gross and microscopic examination and in nine of the animals also by chemical analysis using the method previously described (1).

Results. The data are summarized in tables 1 and 2. They show that deviation of the pancreatic juice from the intestinal tract by means of an external fistula does not produce the fatty infiltration of the liver and the hypolipemia so commonly seen in the insulin treated depancreatized dog.

Seventeen animals survived the complete loss of pancreatic juice for periods ranging from 4 to 50 weeks. The loss of sodium was compensated for by the

TABLE 2

The effect of complete loss of pancreatic juice on liver fat

DOG. NO.	WEEKS AFTER PRODUCTION OF PANCREATIC FISTULA	LIVER FAT PER CENT WET WT.	GROSS AND MICROSCOPIC APPEARANCE OF LIVER	REMARKS
1	12		Normal	Died following biopsy of liver
2	7		Normal	Sacrificed
3	6		Normal	Sacrificed
4	50		Normal	Sacrificed
3	4		Normal	Sacrificed, cannula withdrawn
6	6		Normal	Sacrificed, cannula withdrawn
7	8		Normal	Sacrificed
8	9		Normal	Sacrificed
9	5	6.0	Normal	Sacrificed, cannula withdrawn
10	4	4.0	Normal	Sacrificed
11	6	5.0	Normal	Perforated gastric ulcer
12	4	5.8	Normal	Abscess of thigh, sacrificed
13	6	5.8	Normal	Cannula withdrawn, sacrificed
14	6	6.2	Normal	Cannula withdrawn, sacrificed
15	4	12.1	Slight fat infiltration	Large subphrenic abscess, sacrificed
16	8	8.2	Normal	Cannula withdrawn, sacrificed
17	20	14.7	Slight fat infiltration	Cannula withdrawn, sacrificed

daily intravenous injection of Ringer's solution and the oral administration of alkalies. Death resulted usually from withdrawal of the cannula, infection or perforated ulcer. In every case the pancreas appeared normal, free from infection, and no ducts leading to the duodenum were found. In 14 of the animals the amount of fat in the liver was within the normal range; in 3 an increased amount of fat was found.

Repeated estimations of the serum lipids were made in seven of the fistula animals and recorded in table 1. In general they remained within the normal range. In one case (17) a low value was found on two occasions after 3 months, but the lipids returned to a normal level and remained so for a month before the animal died.

Discussion. The failure of total deviation of pancreatic juice from the

intestine in these experiments to reproduce the fatty infiltration of the liver so commonly seen in insulin treated pancreatectomized dogs indicates that this abnormality is not due to the failure of the pancreatic enzymes or of other substances in the pancreatic secretion to reach the intestine. This finding supports the conclusion of Prohaska, Dragstedt and Harms (2), derived from a similar type of experiment. In this report seven dogs with complete external pancreatic fistulae were described with survival periods of 30, 36, 23, 26, 35, 39 and 43 days. Fatty liver was absent in four of these animals and present to a slight degree in three. Of the latter three, two were found to have extensive subcutaneous abscesses and one died of peritonitis. We have noted on many occasions the association of fatty infiltration of the liver with infections. In the present series one of the animals (15) in which increased liver fat was found had a large subphrenic abscess. If the four cases complicated by extensive infection are excluded there remains a total of 19 animals with complete pancreatic fistulae in the two series with only two in which an increased amount of liver fat (14.7 and 8.2 per cent respectively) developed. These animals survived the loss of pancreatic juice for an average of 8½ weeks.

These findings become more significant when compared with the incidence of fatty infiltration of the liver in depancreatized dogs maintained on the same diet. Thus, of 25 such animals, in a consecutive series, treated with regular insulin, 8 developed a fatty liver in excess of 34 per cent within 30 days, 11 a fatty liver in excess of 25 per cent within 35 days, and the remaining 6 a fatty liver in excess of 14 per cent within 30 days.

The failure of the animals with total pancreatic fistulae to display the marked hypo-lipemia, so commonly seen in depancreatized dogs with the fatty livers of lipocaic deficiency, indicates that this abnormality is also not due to the absence of pancreatic juice from the intestine. This finding is interesting in view of the fact that these animals develop the same bulky stools and steatorrhea seen following complete pancreatectomy. Apparently the decrease in blood fat in the latter case is not due to the impaired absorption from the intestines.

A recent paper by Montgomery, Entenman, Chaikoff and Nelson (6) should be discussed in connection with these experiments. These workers removed the pancreas from eight dogs. The animals were all given a diet low in fat as compared to the one used in our work. Three of the dogs received no supplemental treatment, were sacrificed after 20 weeks, and were found to have fatty livers with a total fatty acid content of 15.1 to 20.5 per cent. Three dogs were fed 250 grams of raw pancreas and 377 cc. of pancreatic juice daily for 8 weeks and thereafter the same amount of pancreatic juice but no pancreas for 20 weeks. At the close of this period the liver fatty acids were found to be 3.7, 7.0 and 5.79 per cent respectively. The remaining two animals were given 250 grams of raw pancreas and pancreatic juice daily for 4 and 3 weeks respectively and then only pancreatic juice for 20 weeks. At the close of this period the liver fatty acids were 3.11 and 3.19 per cent. As a result of this experiment the authors concluded that the fatty livers produced by excision of the pancreas can be completely prevented by the daily ingestion of fresh pancreatic juice.

We believe this conclusion is not warranted for several reasons. In the first place the control is not adequate. The experimental animals were fed large amounts of raw pancreas (250 grams daily) for periods of from 3 to 8 weeks in addition to the pancreatic juice whereas the control animals were given no preliminary treatment with raw pancreas. Secondly, we believe it is necessary to adopt much more rigid criteria before concluding that any given substance, i.e., pancreatic juice or pancreas extract, contains lipocaic. Those employed in this laboratory are described by Dragstedt, Vermeulen, Goodpasture, Donovan and Geer (7). In the present instance a minimum requirement would evidently be to demonstrate that the oral administration of pancreatic juice can relieve a definitely established syndrome of lipocaic deficiency in depancreatized dogs, i.e., cure the fatty liver as checked by biopsy, restore the abnormal glucose excretion and insulin tolerance, relieve the hypolipemia and improve the nutritive state. This is necessary in view of the results with total pancreatic fistula presented in this paper and the finding of Prohaska, Dragstedt and Harms (2) that the oral administration of as much as 1000 cc. of activated pancreatic juice per day could not prevent the development of extreme fatty liver in depancreatized dogs. It seems unlikely that lipocaic present in pancreatic juice would be destroyed in the activated juice since the oral administration of pancreas or pancreas extracts is effective. This fact demonstrates that the active principle in pancreas is not readily destroyed by the digestive enzymes.

CONCLUSIONS

1. The total loss of pancreatic juice from the body by complete pancreatic fistula does not produce the hypolipemia or fatty infiltration of the liver so commonly seen in the insulin treated depancreatized dog.
2. The conclusion that lipocaic is not present in pancreatic juice to any significant extent is confirmed.

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STUDIES ON THE GLYCOGEN METABOLISM OF ATROPHIC AND REGENERATING MUSCLE[1]

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Changes in the concentration of numerous chemical constituents in skeletal muscle undergoing atrophy as a result of denervation can largely be accounted for by progressive changes in the relative amounts of muscle cell and connective tissue phases. Such changes are necessarily limited to those constituents exhibiting an unequal phase distribution in normal muscle. Muscle glycogen presents an important exception to this interrelationship between muscle phases and chemical concentration (1). Following denervation, muscle glycogen undergoes a decrease in concentration at a velocity which markedly exceeds the rate of concomitant phase changes. Moreover, the glycogen of atrophic muscle is not increased by glucose ingestion and is more susceptible than that of normal muscle to the glycogenolytic effects of exogenous adrenalin, insulin and thyroxin (2).

This report is concerned with a search for possible causes of the altered glycogen metabolism exhibited by denervated muscle. The changes in glycogen concentration have been correlated with other chemical and functional changes occurring during the course of atrophy and subsequent regeneration. A comparative study has been made of the glycogen changes in muscles undergoing atrophy from causes other than denervation.

Methods. These studies were carried out on the gastrocnemius muscle of adult albino rats. The general procedure was a concurrent analysis of the corresponding muscles of the two hind limbs, one muscle serving as the control for its contralateral experimental member. Unless otherwise indicated, the data for the atrophic muscles are expressed in percent of their contralateral controls.

Glycogen, creatine, fibrillation, tension and weight measurements were carried out at suitable intervals during the course of atrophy and regeneration following cast application, denervation and tenotomy. Complete denervation of the gastrocnemius was accomplished by crushing the tibial nerve at the level of its junction with the peroneal (3). A total of 215 animals was employed in the denervation studies. The muscles of 74 animals were subjected to tenotomy by clipping off the os calcis, freeing the tendon from all body attachments and sewing the cut end of the tendon to the skin. In 26 animals one hind limb was immobilized by encasement in plaster bandages from the toes to the hip joint. The cast was allowed to harden with the foot in the position of mid-extension. To allow regenerative processes following immobilization, the casts were removed from some animals after being worn for a period of 10 days. Care was taken to

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reject the few animals which showed evidence of circulatory impairment due to ill-fitting casts.

Measurements of muscle creatine, tension and mass were necessarily carried out on animals different from those employed for the glycogen determinations. The methods employed for measuring muscle tension and stimulating muscle and nerve have been described elsewhere (3). The techniques employed for muscle removal, glycogen precipitation and hydrolysis were essentially those described in previous studies (2). The animals chosen for the different experiments were paired as to age, sex and nutritional status.

Fibrillary activity was detected by direct observation of the exposed muscle in reflected light and also by amplification of action potentials led from the muscle through two needle electrodes and visualized on the screen of a cathode ray oscillograph.

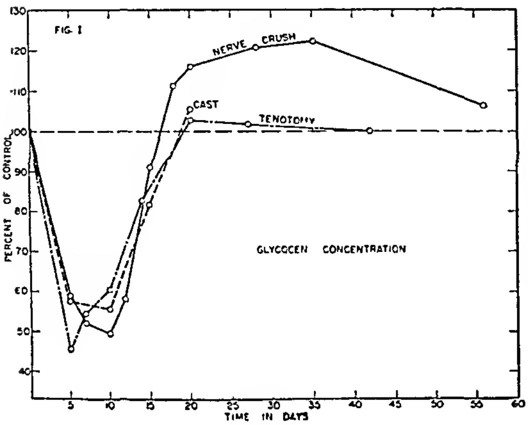
Six animals were subjected to bilateral adrenalectomy and section of the left sciatic nerve at a single operation. These animals then received daily subcutaneous injections of cortical extract (Upjohn) in amounts of 13 rat units per kilogram of body weight. The muscles were removed for glycogen determinations 7 days after the operation.

A study was made of the effects of quinidine administration upon fibrillary activity and muscle glycogen in two groups of animals. To one group of 7 animals the quinidine was administered between 48 and 72 hours after denervation, the time during which the denervated muscle experiences the greatest fall in glycogen concentration. The dosage was 100 mgm. of quinidine sulfate per kilogram of body weight injected intraperitoneally every six hours. We have confirmed the observations of Solandt and Magladery (4) that quinidine in these doses abolished or greatly lessened the fibrillary activity of denervated muscle. In order to minimize glycogen changes in the control muscle due to convulsive states occasionally induced by quinidine, the nerve to the control muscle was sectioned 24 hours prior to the removal of the tissue for analysis. This was considered permissible inasmuch as previous studies (2) have shown that glycogen metabolism is essentially normal in muscle for this period of time after denervation. To another group of 5 animals a single dose of quinidine was administered, followed 2 hours later by 0.75 gram of glucose per 100 grams of body weight given through a stomach tube. The animals were sacrificed 3 hours after glucose administration and the muscles analyzed for glycogen.

The sciatic nerves to both limbs were sectioned in 5 animals. The denervated gastrocnemius of one limb was subjected to electrical stimulation under light ether anesthesia for a period of 3 minutes twice daily. The stimuli consisted of 60 strong induction shocks per second and were applied to the muscle through needle electrodes which pierced the skin. The non-stimulated denervated muscle of the contralateral limb served as a control. Muscles were removed for glycogen determinations 78 hours after denervation and 8 hours after the last period of stimulation. Similar experiments were carried out on 6 animals in which 70 hours were allowed for degeneration of the severed nerve before commencement of electrical stimulation. Muscles were removed for glycogen de-

terminations 126 hours after denervation and 8 hours after the last period of stimulation.

RESULTS. Denervation, immobilization by casts and tenotomy were followed in a few days by comparable marked falls in the glycogen concentration of the affected muscles (fig. 1). The decrease in glycogen concentration occurred at



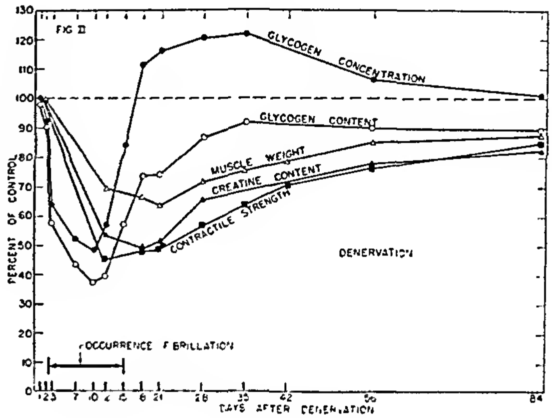
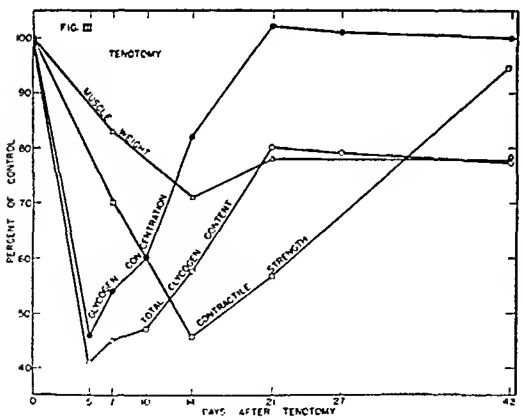


Fig. 1. A comparison of the changes in muscle glycogen concentration following cast application and removal, denervation and tenotomy.

Fig. 2. The relation of glycogen changes to other phenomena in skeletal muscle following denervation by nerve crush.



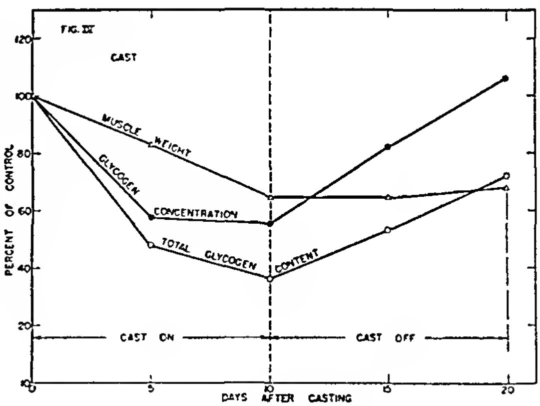


Fig. 3. Average values for the changes in muscle weight, strength and glycogen stores during atrophy and regeneration following tenotomy.

Fig. 4. Illustrating the rates of change in muscle weights and glycogen levels following immobilization by cast and subsequent cast removal.

a much greater velocity than the loss of muscle creatine, contractile strength or weight (figs. 2, 3 and 4). Conversely, restoration of muscle function through regeneration of the crushed nerve, regrowth of the tendon attachment and removal of the cast was associated with a rapid and complete restoration of glycogen concentration to normal levels (fig. 1). This occurred at a higher velocity

than the recovery of muscle mass, strength or creatine concentration (figs. 2, 3 and 4). At certain times during the course of regeneration, the muscles previously subjected to denervation exhibited glycogen concentration values considerably above those found in their contralateral controls. Significant increases above normal values were not observed in the muscles recovering from tenotomy.

Fibrillary activity first appeared on the second or third day after nerve crushing and disappeared on about the fifteenth day, a time shortly after the nerve had undergone sufficient regeneration to cause upon stimulation the development of measurable amounts of tension in its muscle. Fibrillary activity was not detected in either tenotomized or casted muscle.

TABLE 1

A summary of the effects of adrenalectomy, electrical stimulation and quinidine upon the glycogen concentration in control and denervated muscle

CONDITION OF ANIMAL	HOURS AFTER DENERVATION	GLYCOGEN CONCENTRATION*		GLYCOGEN CONCENTRATION AS PER CENT OF CONTRALATERAL CONTROL
		denervated	control	
Adrenalectomized	168	326	546	60
Control	168	350	670	52
Quinidine	72	240	422	57
Control	72	414	647	64
Quinidine + glucose	72	306	753	41
Control	72	378	740	51
Electrical stimulation	78	490		178
Control**	78	276		
Electrical stimulation	126	494		182
Control**	126	271		

* Milligrams per 100 grams. muscle expressed as glucose equivalent.

** Control was denervated non-stimulated contralateral muscle.

Administration of quinidine in doses sufficient to abolish fibrillary activity failed to prevent the usual fall in glycogen concentration following denervation (table 1). Moreover, the afibrillary quinidinized muscle did not experience an increase in its glycogen values following glucose ingestion. In this respect it behaved like denervated muscles in animals not subjected to quinidine administration. It should be pointed out that glucose ingestion resulted in an appreciable increase of glycogen storage in the control muscles of both quinidinized and and non-quinidinized animals. The animals subjected to adrenalectomy and sustained by adequate doses of adrenalin-free cortical extract experienced essentially the same drop in the glycogen concentration of their denervated muscles as did control non-adrenalectomized animals (table 1). Electrical stimulation of denervated muscle resulted in higher glycogen concentrations than existed in

the contralateral non-stimulated denervated control muscle. The stimulation was effective when initiated either immediately after denervation or after complete degeneration of the motor nerve (table 1).

Discussion. The glycogen concentration in skeletal muscle proved to be a sensitive indicator for the onset of changes characteristic of atrophy and regeneration. The decrease in glycogen concentration in denervated muscle exceeded in velocity the loss of creatine, mass or strength. Conversely, the onset of functional reinnervation was accompanied by a rapid and complete restoration of glycogen concentration which occurred sooner than the recovery of muscle mass, strength or creatine concentration.

An occurrence of essentially the same degree of glycogen loss from the denervated muscles of adrenalectomized animals as from the denervated muscles of control animals indicates that the cause for the disturbance in glycogen metabolism cannot be attributed to the glycogenolytic action of endogenous adrenalin on a tissue sensitized by denervation.

The time of onset for the rapid decline in glycogen concentration was concomitant with a loss of excitability in the peripheral portion of the crushed nerve and the appearance of fibrillary contractions in the denervated muscle. The subsequent recovery of glycogen concentration to normal or above normal values occurred at a time when the regenerating nerve upon stimulation was capable of eliciting a measurable amount of tension in its muscle. This time for the glycogen recovery also approximated the time for the disappearance of fibrillary activity from the muscle. Time relationships for the above changes strongly suggest that the depletion of glycogen may be due to the effects of fibrillary activity. Our experiments, however, offer three lines of evidence against such a cause and effect relationship. The finding that quinidine administration was able to abolish fibrillary activity without preventing the usual fall in glycogen concentration is considered to be evidence against the idea that the low glycogen concentration in denervated muscle is due to "overwork" or exhaustion from fibrillary activity. The finding that electrical stimulation of the paralyzed muscle resulted in higher glycogen levels rather than further decreases likewise argues against the "overwork" theory. Furthermore, the finding of equally rapid decreases in muscle glycogen levels following immobilization by casts and tenotomy, conditions in which fibrillary activity is absent, strongly suggests that the causes for the altered glycogen metabolism must be sought elsewhere.

The striking similarities in time relationships for the glycogen changes in three types of muscle atrophy and subsequent regeneration suggest that the causes for such may reside in some basic factor common to denervation, immobilization and tenotomy. It is suggested that this factor may be a decrease in metabolism associated with a reduced development of tension by the muscles. In each of the atrophic conditions there was a decrease in the ability of the muscle to produce tension and the metabolism associated therewith. Reflex tension was abolished by denervation and greatly lessened by the loss of the stretch stimuli to the proprioceptive receptors after immobilization and tenotomy. It appeared quite possible that the absence of normal muscle contractions resulted in insuffi-

cient energy levels for the coupled reactions that may be required for a normal rate of glycogen synthesis (5). The progressive resumption of normal types and degrees of activity following reinnervation and release from immobilization would provide the energy conditions favorable for glycogen synthesis at rates adequate for the establishment of pre-atrophy levels. It may be presumed that by electrical stimulation of paralyzed muscle the necessary energy for maintenance of a higher concentration of glycogen than existed in the non-stimulated denervated muscles becomes available. It is not improbable that energy level factors may be involved in the loss of protein from muscle during atrophy and its recovery during the return of normal functional states. The observation of Fischer (6) that electrical stimulation delays the atrophy of denervated muscle is significant in this respect.

It should be pointed out that another basic factor may be common to the different types of atrophy. That is the effects of the loss, partial or complete, of neurohumoral agencies acting in a trophic manner, apart from the rôle they may play in the excitation processes associated with tension development. However, the finding that artificial stimulation of the muscle will arrest the weight loss and fall in glycogen concentration after the disappearance of viability in the severed nerves does not suggest any important rôle of such agencies other than that concerned with stimulus transmission.

SUMMARY

Experiments were carried out on the gastrocnemius muscles of adult albino rats. Denervation, immobilization by casts and tenotomy were followed in a few days by comparable marked falls in the glycogen concentration of the affected muscles. The glycogen changes occurred at a greater velocity than the loss of creatine, mass or strength from atrophic muscles. Conversely, restoration of muscle function through nerve regeneration, regrowth of tendon and cast removal was followed by a rapid and complete restoration of glycogen concentration to normal levels sooner than the recovery of creatine, mass or strength.

Adrenalectomy did not prevent the fall in glycogen concentration in denervated muscle.

Even though the fall in glycogen concentration and subsequent recovery coincides in time with the appearance and disappearance of fibrillary activity, it is believed that fibrillary contractions per se are not the cause of the low glycogen concentration in denervated muscle. The finding of equally marked and rapid decreases in the glycogen concentration of muscle undergoing atrophy without fibrillary activity, the fact that quinidine abolishes fibrillary activity without preventing the fall in glycogen and the observation that electrical stimulation will lessen rather than enhance the loss of glycogen from denervated muscle, indicate fundamental causes other than fibrillary activity.

It is suggested that the absence of tension development in the atrophic muscle results in insufficient energy levels for the coupled reactions which may be required for glycogen synthesis.

We are grateful to Dr. G. Clinton Knowlton for assistance in the fibrillary activity studies.

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POSTURAL CHANGES IN RESPIRATION[1]

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Dependence of respiration on body position was recognized by Liljestrand and Wollin in 1913. They demonstrated an increased ventilation during standing which they attributed chiefly to an alteration in respiratory rate. McMichael (1937) believes the lowering of alveolar CO_2 in the erect position found by him and others (Turner, 1927; Main, 1937) refutes the possibility that the increase in pulmonary ventilation is apparent only, secondary to a postural change in pulmonary capacity. Neither is the increased metabolism of standing adequate to account for the augmentation (Turner, 1927; Hamilton et al., 1932).

There are many indications that postural changes in respiration are related to the hydrostatic opposition offered the circulation in standing. Turner (1927) noted that overbreathing occurred especially among subjects who tolerated protracted standing poorly. This observation is supported by the postural studies of McMichael (1937) in which the pulmonary ventilation per 100 cc. of O_2 consumed varied approximately inversely as the absolute cardiac output. Hamilton et al. (1932) report that an increase in pulmonary minute volume became evident as their male subjects reached an angle of 50 degrees from the horizontal when supported on a tilting table and moved vertically by 10 degree increments. Approximately the same angle was found by Hellebrandt and Brogdon (1938) to be the critical position at which circulatory embarrassment commonly become evident in young women. This study is concerned with systematic observations of respiratory behavior under conditions of graded hydrostatic handicap to the circulation.

METHODS. The report deals in part with a further analysis of data obtained in a study of the postural metabolism of three young adult women (Hellebrandt et al., 1940). Pulmonary ventilation was determined from respiratory rate and amplitude as obtained in closed circuit indirect calorimetry. It was converted into liters per minute under standard conditions. To exclude the effect of oxidative processes on ventilation values, the "ventilation equivalent of oxygen" was calculated. The ratio, as used by Anthony (1930) and others (Knipping and Moncrieff, 1932; McMichael, 1937), represents the quantity of air from which 100 cc. of O_2 are removed in the lung, or $\frac{PV \times 100}{cc.\ O_2/min.}$. It is similar to Simonson's "caloric ventilation equivalent" (1926) and Herbst's "utilization coefficient" (1928).

[1] Supported in part by the Wisconsin Alumni Research Foundation and the Council of Physical Therapy of the American Medical Association.

The three subjects of the major observations, young adult women, were under strictly basal conditions. After repeated 5-minute observations in recumbency, a series of determinations in some vertical or semi-vertical posture was made. Observations followed each other with a brief interruption after each 5-minute run during which pressure from the nose clip was momentarily relieved and the spirometer was partially refilled. As many as twelve consecutive runs could be made on a trained subject without restlessness or discomfort. The hydrostatic handicap was graded by the use of a tilting board. It was nullified by suspending the subject from the head and shoulders in a 180 gallon tank filled with water kept at body temperature. The full effect of gravity was observed in supported standing, with the subject immobilized by an arrangement of padded supporting bars, and in unsupported standing during which free movement above a stationary base was allowed as dictated by involuntary postural sway. Heart rates were counted for 30 seconds at minute intervals by pre-cordial auscultation. The experimental periods varied from 10 to 60 minutes in length.

The respiratory response to protracted support in the critical position (75 degrees from the horizontal) was studied in 22 healthy young adult women, well accustomed to the procedures. These experiments were not performed in the post-absorptive state but were basal in all other respects. Room temperature was held approximately at 75 degrees F. After horizontal observations were made, the subject was shifted to the semi-vertical position and rested motionless, leaning against the tilting table. Measurement of O_2 consumption was resumed within three minutes after the change in posture and was continued for 40 minutes or until the subject presented the signs of pre-collapse or actual syncope.

Results and their interpretation. Two of the three subjects upon whom the most extensive data were obtained demonstrated marked respiratory alterations with the changes in posture. The third subject (R. H. T.) exhibited some increase in ventilation as the hydrostatic handicap to the cirulation was augmented. It was never marked. Observed differences were in some positions so slight as to be statistically unreliable. The more exaggerated respiratory activity of subjects F. A. H. and R. E. B. parallels their cardiovascular response to passive support at the 75 degree angle. F. A. H. could tolerate only 27 minutes in that position, with a sudden slowing of heart rate after an acceleration to 123 beats per minute preceding syncope. R. E. B. could tolerate 45 minutes without syncope, but only with a marked progressive increase in heart rate.

1. *Graded degrees of passive support.* A progressive increase in total ventilation for R. E. B. is evident in figure 1. It appears to be the result of an augmenting rate of breathing. Table 1 gives the mean of the values obtained at each angle of support for the three subjects. Treated statistically, the differences between the adjacent means from 15 degrees through 75 degrees were reliable throughout for R. E. B. and F. A. H. Respiratory amplitude was affected less consistently than was rate although deep inspirations and yawns did appear at the more vertical angles. The increasing ventilation equivalents (table 1) indicate that the magnitude of the respiratory change is out of proportion to

concomitant increases in oxygen consumption. This is statistically true for the three subjects in most instances.

All the respiratory phenomena increase little further or fall off when the posture changes from 75 to 90 degrees from the horizontal. This was found to be true also in regard to the behavior of the heart, suggesting that muscle action consequent upon more active support of the body weight in full verticality exerts sufficient pressure upon the vascular walls to oppose in some measure a progressively greater accumulation of fluid in the dependent limbs due to the combined effects of stagnation and edema.

2. *Protracted support in a near-vertical position.* In the group of 22 young women there was a wide range in immediate response to change from the horizontal to a 75 degree angle. That the majority of the values for ventilation were in magnitude and direction influenced in the first 5 minutes by variations in oxygen consumption is indicated by the fact that in only four cases did the ventilation equivalent alter by more than 10 per cent. In these four, the ventilation equivalent increased between 16.9 and 43.1 per cent, evidence of definite over-ventilation.

TABLE 1

*Respiratory behavior of subjects supported at progressively more vertical angles**

SUBJECT		DEGREE OF DEVIATION FROM THE HORIZONTAL					
		15	30	45	60	75	90
F. A. H.	PV	6.87	7.44	8.37	10.06	12.10	11.68
	VE	3.75	4.01	4.53	5.14	5.84	5.66
	RR	19.90	22.78	24.94	24.75	28.95	25.55
	RA	346.82	328.00	337.15	411.15	420.80	450.95
R. E. B.	PV	6.06	7.29	7.93	8.37	9.29	9.59
	VE	3.42	4.10	4.22	4.65	5.12	5.14
	RR	15.10	19.22	23.05	23.04	25.25	25.17
	RA	402.30	381.32	345.31	366.70	372.40	384.73
R. H. T.	PV	5.74	6.19	6.33	6.47	7.22	7.10
	VE	2.93	3.18	3.28	3.27	3.44	3.47
	RR	12.20	13.40	14.17	13.69	14.30	14.90
	RA	474.70	464.20	451.60	474.70	509.90	478.60

* Values are the mean of from 8 to 12 five-minute determinations.
PV: Pulmonary ventilation in liters per minute.
VE: Ventilation equivalent (liters per 100 cc. of O_2 used).
RR: Respiratory rate per minute.
RA: Respiratory amplitude in cubic centimeters.

During the period of support at the 75 degree angle, pulmonary ventilation in 20 of the 22 subjects rather steadily increased. In nine cases the increase was due primarily to accelerated respiratory rate, in seven chiefly to greater amplitude, and in four to a combination of both factors. Fourteen of the subjects demonstrated increases so out of proportion to changes in metabolism

that final ventilation equivalents from 10 to 61 per cent higher than in the horizontal were obtained.

3. *Vertical postures.* Table 2 summarizes the data for all strictly vertical positions and figure 1 illustrates the response of R. E. B. With *suspension in water*, respiratory behavior approximates that in the horizontal position or is somewhat reduced. This suggests that the hydrostatic effect of gravity on the circulation is a factor which can modify respiration, for when it is nullified, the vertical position alone has no influence on ventilation. Undoubtedly, the withdrawal of proprioceptive stimuli and the exclusion of variable environmental influences contribute materially to the lessening.

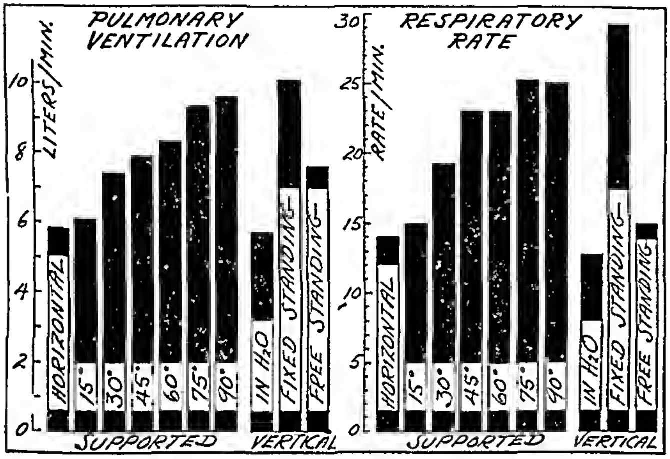

Fig. 1

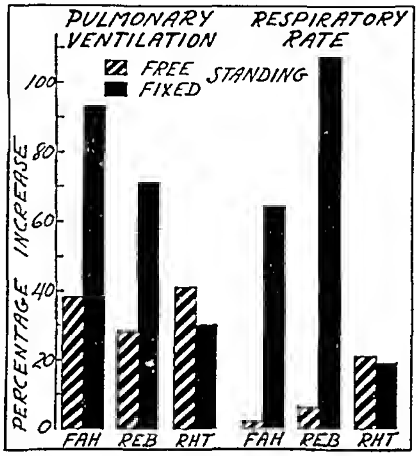

Fig. 2

Fig. 1. Respiratory response of one subject (R. E. B.) in supported (angle of tilt from the horizontal given), unsupported, and suspended postures. Columns are based on the means of 8 to 14 five-minute determinations in semi-vertical or vertical positions and are compared with the mean of 65 determinations in the horizontal.

Fig. 2. Comparison of effect on respiration of two types of vertical posture, showing percentage increase over horizontal values for pulmonary ventilation and respiratory rate. Columns are based on the means of 11 to 23 five-minute determinations and are compared with the mean of 93, 65 and 77 determinations for the three subjects in the horizontal.

Deprivation of postural sway in the erect position markedly augmented pulmonary ventilation in subjects F. A. H. and R. E. B. They showed, respectively, increases of 92.8 and 71.1 per cent over the horizontal values, figures definitely beyond any expected rise due to the metabolic cost of standing as is apparent from the significant increase in ventilation equivalents (table 2). The change is largely brought about by alteration in rate of breathing as illustrated in figure 2.

Standing comfortably and making no effort to restrict involuntary movement above the stationary base of support, subjects F. A. H. and R. E. B. exhibited mean pulmonary ventilation increases of 36.1 and 28.8 per cent over their horizontal values. The relatively slight change in ventilation equivalents indicates that such increases in ventilation are partially related to additional oxygen utilization. R. H. T. demonstrated comparatively little augmentation

of respiration in either posture and that amount is shown to be related to a concomitant rise in oxygen consumption. The ventilation equivalents do not differ significantly from those in the horizontal position.

Respiratory irregularities were prominent in the records of the two subjects who showed heightened respiration in the erect position. Deep inspirations and yawns were interspersed from one to eight times during a 5-minute period, more frequently in the rigid posture than when physiological sway was permitted. Deprived of sway, the subjects exhibited a periodicity in amplitude

TABLE 2

*Respiratory behavior in different vertical positions compared with that in the horizontal**

SUBJECT	F	VENTILATION EQUIVALENT, LITERS/100 CC. OF OXYGEN			PULMONARY VENTILATION, LITERS/MIN.			RESPIRATORY RATE PER MINUTE			RESPIRATORY AMPLITUDE, CC.		
		Mean	σ_{dist}	$D/\sigma_{diff.}$	Mean	$\sigma_{dist.}$	$D/\sigma_{diff.}$	Mean	$\sigma_{dist.}$	$D/\sigma_{diff.}$	Mean	$\sigma_{dist.}$	$D/\sigma_{diff.}$
F. A. H.													
Basal	93	3.93	0.33		6.98	0.95		21.70	3.35		342.79	19.10	
Water	12	3.70	0.20	3.65	7.38	0.50	2.27	20.25	1.48	2.64	364.65	12.63	1.98
Fixed	11	7.14	1.32	8.07	13.66	2.19	10.01	35.70	4.70	9.60	388.86	38.86	3.65
Sway	23	4.49	0.88	3.04	9.50	0.78	13.33	22.08	0.96	0.95	430.10	27.31	14.49
R. E. B.													
Basal	65	3.36	0.27		5.89	0.28		14.16	1.30		419.48	39.00	
Water	10	2.90	0.19	6.57	5.70	0.22	2.47	12.76	1.14	3.54	449.80	33.41	1.98
Fixed	12	5.43	0.58	12.18	10.13	1.30	11.28	29.28	4.92	10.59	448.22	15.67	4.34
Sway	14	3.57	0.31	2.36	7.63	0.59	10.81	15.09	2.10	1.60	509.84	54.23	5.91
R. H. T.													
Basal	77	3.07	0.31		5.79	0.54		11.63	1.75		504.69	61.92	
Water	11	2.61	0.16	8.52	5.79	0.39	0	12.03	1.30	0.91	486.10	58.60	0.98
Fixed	12	3.37	0.40	2.54	7.51	0.55	10.12	13.90	1.39	5.07	541.67	40.61	2.72
Sway	12	3.28	0.40	1.78	8.15	0.76	10.31	14.18	1.84	4.50	586.37	34.18	6.72

* Water: Suspended in 180 gallon tank of water.

Fixed: Immobilized in standing position by extrinsic supports.

Sway: Free postural sway unrestrained.

F: Number of 5-minute periods of observation.

$D/\sigma_{diff.}$: Comparison of mean of that position with the horizontal basal mean. It is customary to take a $D/\sigma_{diff.}$ of 3 as indicative of a significant difference.

occasionally so marked as to resemble Cheynes-Stokes respiration. Syncope when it occurred always followed one of these exaggerated phases.

Comment. Respiratory movements are sometimes considered as a subsidiary line of defense against orthostatic collapse by virtue of expiratory compression of the abdomen and inspiratory thoracic suction (Best and Taylor, 1939). Hill and Barnard in 1897 showed that when splanchnic vasomotor tone of their animals was destroyed, respiratory efforts maintained the circulation, though far less efficiently. On the basis of plethysmographic experiments with dogs

in 1933, Eyster and Hicks believe that the influence of the extent of breathing on venous return is not as great as is ordinarily stated. More recently, Boyd and Patras (1941), using a cardiometer in the closed chest, have reported increases in diastolic and stroke volume with inspiration which were exaggerated by the deep and prolonged breathing following vagotomy.

In our experiments it is impossible to evaluate the importance of this respiratory pump as an aid to the circulation. Respiratory behavior is shown to be closely related to cardiovascular competence. It is augmented as the gravitational handicap to the circulation is made greater and approaches normal when the compensatory action of postural sway reduces that handicap. With few exceptions both fainters and non-fainters responded with some augmentation of ventilation to protracted support in near-verticality. However, the point of failure of the circulation did not appear to bear any time relationship to the percentage increase in ventilation. Whether the increase in breathing prolongs the period of tolerance for the erect position through aspiratory action can only be a matter of conjecture. The fact that the increases observed were in many cases due to rises in rate with reductions in ampliture renders this unlikely. The augmented ventilation seems rather a reflection of the general slowing of the circulation in which the medullary centers are more or less seriously deprived of oxygen unless more effective compensatory mechanisms combat the pull of gravity upon the blood column. Of these, the rhythmic squeezing action of muscle is clearly important.

CONCLUSIONS

1. Respiratory changes induced by alteration of posture outstrip concomitant metabolic increases as verticality is approached.
2. Increases in ventilation in vertical postures appear to be secondary to interference with blood flow to the head.
3. Postural sway is a factor of material benefit in combating orthostatic hyperpnea.

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LIVER FUNCTION, PULSE RATE AND TEMPERATURE OF HYPERTHYROID DOGS

EFFECT OF A YEAST-FREE DIET AND A HIGH B VITAMIN DIET[1, 2]

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Previous studies on the liver function of dogs during hyperthyroidism have shown that a deficiency of the B vitamins is related to the production of an abnormal liver function. It was found that the removal of the yeast from the diet of hyperthyroid dogs was followed by the sudden appearance of abnormal liver functions, as judged by the excretion of injected bromsulphalein (1, 2). Once an abnormal liver function had been produced in hyperthyroid dogs by removal of the yeast from the diet, intensive treatment with crystalline vitamin B_1 (thiamine) and a yeast concentrate would not restore the liver function to normal (2).

Thyroid feeding also produced a marked tachycardia, but on removal of the dietary yeast a rapid fall in pulse rate was observed, the pulse rate falling to as low as 80 beats per minute while still feeding thyroid gland (2). The injection of crystalline thiamine would raise the pulse rate to the hyperthyroid level in 24 to 48 hours. While the mechanism of this effect of thiamine on pulse rate during hyperthyroidism is unknown, it is evident that the tachycardia observed in experimental hyperthyroidism depends not only on thyroid feeding per se but also on an adequate intake of thiamine. The treatment of these dogs with crystalline thiamine and a yeast concentrate would maintain the pulse rate at the previous hyperthyroid level, but after 90 to 100 days of thyroid feeding the pulse rate again began to fall toward normal. The pulse rate remained above normal, but not at the previous hyperthyroid level (2).

Further information on the interrelationships between the B vitamins and the thyroid gland was obtained by studying *a*, the effect of thyroid feeding on the liver function and pulse rate of dogs receiving a diet rich in the B vitamins from the start of thyroid feeding; *b*, the effect of thyroid feeding on the liver function and pulse rate of dogs receiving a vitamin-B-complex-free diet.

METHODS. As a sex difference in response to thyroid feeding has been reported in rats (3), only male dogs were used. Thyroid gland[3] was fed in a dosage of

[1] The authors wish to thank Eli Lilly and Company for a grant in support of these experiments.

[2] This work was done in the laboratory of Dr. W. W. Swingle and we are indebted to him for the necessary facilities to undertake this work.

[3] The authors are indebted to Dr. C. N. Fry of The Fleischmann Laboratories for supplying the yeast concentrate, to Dr. R. T. Major of Merck and Company for supplying the crystalline thiamine, and to Dr. H. W. Rhodehamel of Eli Lilly and Company for the desiccated thyroid gland.

0.4 or 0.6 gram per kilogram of body weight per day (table 1) throughout the experiment. The dogs were fed a modification of Cowgill's casein diet no. III which is free of the B vitamins (1). The animals were fed at the same time each day, being allowed to eat as much as they wanted for a three hour period, and the food intake measured. Control measurements were made for a period of 3 to 4 weeks, using a yeast supplement to the basal diet, until the food intake became fairly constant. Liver functions were determined by the bromsulphalein method of Rosenthal and White as previously described (2), using 5 mgm. of dye/kgm. body weight.

Four of the dogs were fed 10 grams of a yeast concentrate[3] per day, the yeast containing 200 U.S.P. units of vitamin B_1, 230 gamma of riboflavin, 200 gamma of vitamin B_6, and 1500 to 2000 gamma of calcium pantothenate per gram. Two of these dogs also received a daily injection of 1.0 mgm. of thiamine each day. The administration of the yeast concentrate and thiamine began with the feeding of thyroid gland and was continued throughout the experiment.

Three other dogs were fed thyroid gland, but at the start of thyroid feeding

TABLE 1

DOG NO.	WEIGHT	THYROID GLAND FED PER DAY PER KGM. BODY WEIGHT	YEAST CONCENTRATE FED PER DAY	THIAMINE INJECTED PER DAY
	kgm.	*grams*	*grams*	*mgm.*
1	16.1	0.6	10	0
2	8.8	0.6	10	1.0
3	10.1	0.4	10	1.0
4	12.8	0.4	10	0
5	10.3	0.4	0	0
6	8.6	0.6	0	0
7	10.8	0.6	0	0

the yeast was removed from the diet, and they were fed the yeast-free diet throughout the period of thyroid feeding.

RESULTS. *Liver function.* The control measurements of liver function did not show a retention of dye greater than 12 per cent at the end of 30 minutes. A dye retention of 15 per cent or greater was therefore regarded as abnormal. Of the four hyperthyroid dogs receiving the high yeast diet, dog 3 first showed an abnormal liver function on the 73rd day of thyroid feeding. Later, an abnormal liver function developed in dogs 2, 4 and 1 on the 84th, 91st and 112th day of thyroid feeding respectively (fig. 1). Figure 1 shows the time and degree of dye retention when an abnormal liver function was first observed. The dye retention of the dogs later increased and remained between 30 to 50 per cent retention of dye. No difference was observed in the production of the abnormal liver functions between the dogs fed 0.4 or 0.6 gram of thyroid per kilogram of body weight. The amount of B vitamins supplied to these dogs was sufficient to maintain food intake and prevent a loss of body weight. Included in figure 1 for comparison are three dogs fed the same diet but receiving

only a small amount of yeast (2). The amount of yeast fed (basal yeast) is sufficient for normal maintenance, but when the requirements for the B vitamins are increased by thyroid feeding a relative deficiency of the B vitamins is produced. The thyroid-fed dogs receiving basal yeast supplements developed an abnormal liver function in an average of 45 days, whereas the hyperthyroid dogs fed a high B vitamin diet showed abnormal liver functions after an average of 90 days. This demonstrates, therefore, that feeding hyperthyroid dogs a diet rich in the B vitamins will delay the onset of an abnormal liver function, as judged by the bromsulphalein test, but will not prevent its appearance.

The hyperthyroid dogs receiving a yeast-free diet showed a marked loss of appetite and weight and rapidly developed abnormal liver functions (figs. 1, 4), the average time being 22 days for the first appearance of abnormal dye retention.

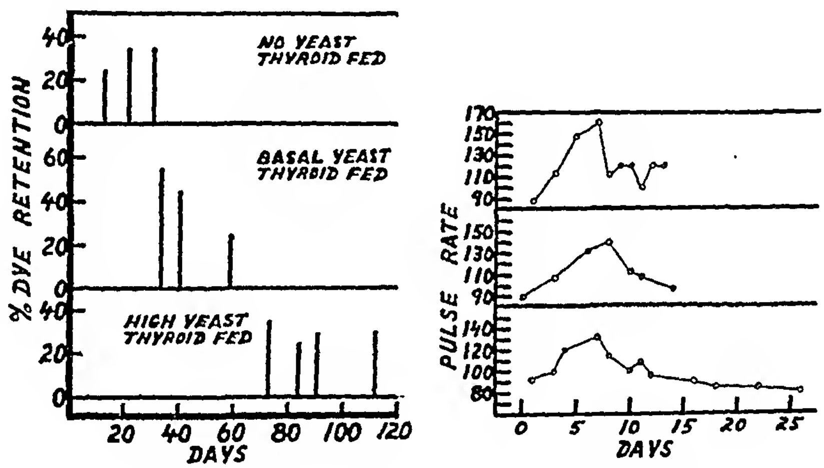

Fig. 1 Fig. 2

Fig. 1. Liver functions of hyperthyroid dogs fed various amounts of B vitamins. The graph illustrates the time at which an abnormal liver function was first observed in each dog and the percentage of dye retention at that time.

Fig. 2. The pulse rate of hyperthyroid dogs fed a yeast-free diet. Note the rapid rise in pulse rate which returns to normal within 10 days of thyroid feeding, as soon as the body stores of thiamine are depleted.

tion. Thus the feeding of a diet free of the B vitamins renders the dogs extremely susceptible to the effects of thyroid feeding, producing an abnormal liver function in a short time.

Pulse rate. It has previously been shown that the tachycardia typically seen in experimental hyperthyroidism cannot be maintained if the yeast is removed from the diet after about 30 to 50 days of thyroid feeding (2). An injection of vitamin B_1 (thiamine) will raise the pulse rate to its previous hyperthyroid level (2). Confirmation of this result was obtained when the yeast was removed from the diet at the time thyroid feeding was started. When this was done only a short, rapid rise in pulse rate was obtained, which returned to normal during the first 10 days of thyroid feeding (fig. 2). The small initial rise in pulse rate is due to the presence of the body stores of thiamine, which were soon

exhausted, and the pulse rate declined to normal, even while still feeding thyroid gland. Thus, on a diet free of the B vitamins, thyroid feeding produced only a temporary rise in pulse rate, which returned to normal in 10 days. The tachycardia seen in experimental hyperthyroidism is therefore not due to thyroid feeding per se, but also depends on an adequate supply of thiamine (2).

Earlier studies have shown that the low pulse rate produced in hyperthyroid dogs by removing the yeast from the diet can be raised to its previous hyperthyroid level by injecting thiamine (2). It was also observed that intensive treatment with thiamine and yeast concentrate maintained the tachycardia,

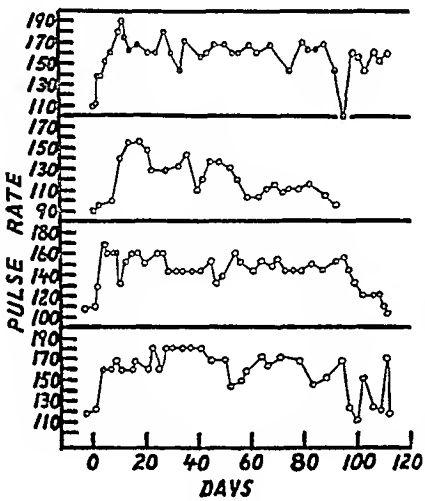

Fig. 3

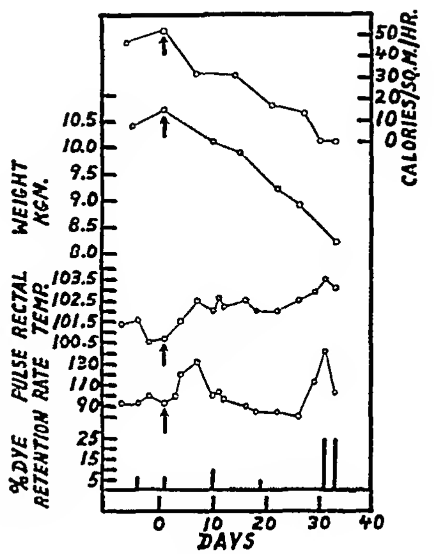

Fig. 4

Fig. 3. The pulse rate of hyperthyroid dogs fed a high B vitamin diet, on which the tachycardia produced by thyroid feeding cannot be maintained indefinitely. The fall in pulse rate towards the end of the experiment seems to be associated with a compensatory hypertrophy of the heart.

Fig. 4. Composite graph of dog 7 receiving thyroid and a yeast-free diet. Note the rapid loss of appetite and weight, the continuous rise in rectal temperature, and a terminal rise in pulse rate. The food intake is calculated as calories consumed per square meter of surface area per hour.

but only for 90 to 100 days, after which time the pulse rate began to fall towards normal (2). This was explained as probably being due to a compensatory hypertrophy of the heart. This was further studied in the dogs that were fed a diet rich in the B vitamins at the time thyroid feeding was started. The results (fig. 3) showed that the tachycardia in hyperthyroid dogs can not be maintained indefinitely by such treatment. Three of the dogs showed a drop in pulse rate as the thyroid feeding was continued. The fourth dog would probably have shown a similar drop in pulse rate had the thyroid feeding been continued longer.

At necropsy the heart of the hyperthyroid dogs receiving the high B vitamin

diet was found to be hypertrophied, particularly the left ventricle. The heart of these dogs weighed an average of 97.7 grams per 10 kilos of body weight as compared with 70.8 grams per 10 kilos of body weight of normal dogs (table 2). The hyperthyroid dogs receiving a yeast-free diet also showed some cardiac hypertrophy, the heart weight being 85.0 grams per 100 kilos of body weight. These dogs, however, were fed thyroid gland for a shorter period of time.

Rectal temperature. The rectal temperature of hyperthyroid dogs receiving a high yeast diet was maintained at a level above normal throughout the experiment. Dog 4 showed an increase in rectal temperature from a normal of 101.0°F. to 102–102.5°F. after thyroid feeding was started. Likewise dog 3 increased from a normal of 100.5–101.0 to 102.0°F. and dog 1 from 101.5 to 102–102.5°F. These high temperatures were maintained fairly constant throughout the experiment. Dog 2 was not as constant. His rectal temperature rose from 101.5 to 102–102.5°F., returning at intervals to normal.

The hyperthyroid dogs fed a yeast-free diet showed a greater rise in rectal

TABLE 2

Weight of heart of hyperthyroid dogs

(Grams per 10 kilos of body weight)

DOG NO.	HYPERTHYROID HIGH YEAST DIET	DOG NO.	HYPERTHYROID YEAST-FREE DIET	DOG NO.	NORMAL DOGS
1	80	5	72	8	70
2	100	6	94	9	70
3	93	7	86	10	59
4	76			11	78
				12	77
Average.....	97.7		85.0		70.8

temperature (fig. 4). A marked terminal rise in temperature was also seen as the dogs developed anorexia and lost weight. A terminal rise in pulse rate was also seen in one dog (fig. 4).

Discussion. Thus far the B vitamins have been shown to bear a causal relationship to the production of an abnormal liver function in hyperthyroid dogs (1, 2). After an abnormal liver function has been produced in hyperthyroid dogs it cannot be brought again to normal by intensive treatment with thiamine and yeast concentrate (2). In this paper it was shown that an abnormal liver function develops very rapidly, in an average of 22 days, when thyroid-fed dogs receive a yeast-free diet. Hyperthyroid dogs fed a basal amount of yeast each day developed an abnormal liver function in an average of 45 days. In other hyperthyroid dogs receiving a high B vitamin diet it took an average of 90 days before an abnormal liver function was produced (fig. 1). This demonstrates that a direct relationship exists between the amount of B vitamins in the diet of a hyperthyroid dog and the time elapsed before an abnormal liver function develops. With a diet rich in the B vitamins the pro-

duction of an abnormal liver function can be greatly delayed, but cannot be prevented. Enough B vitamins were administered to these dogs to prevent a loss of weight and to maintain the food intake at the hyperthyroid level. Whether a greater intake of the B vitamins would further have delayed the appearance of an abnormal liver function is not known. It may be said, however, that the development of an abnormal liver function does not depend on a loss of weight. In order to maintain body weight, hyperthyroid dogs fed yeast concentrate and injected with thiamine voluntarily eat approximately 1.5 times as many calories per day as do normal dogs (4). It is a possibility that the increased food intake, which in turn will provide more metabolic work for the liver, is a factor in itself which will tend to produce an abnormal liver function. Unpublished work on serum phosphatase has shown that the phosphatase remains normal while dye retention is normal, and that an increase in phosphatase is only obtained when the dye retention is abnormal.

Bartels (5) has reported that in hyperthyroid patients no correlation was found between the duration of the disease and the incidence of abnormal liver functions. Rather, a normal liver function was generally obtained in patients who had no previous history of a loss of weight. Judging from the work on hyperthyroid dogs it is possible that the hyperthyroid patients not showing a weight loss were receiving a greater supply of the B vitamins, which would also aid in maintaining a normal liver function. Further references on liver function in Grave's disease may be found in Boyce's recent monograph on the liver (6).

The effect of the high B vitamin diet in delaying the appearance of the abnormal liver function in hyperthyroid dogs is in agreement with other investigations. It has been reported that the administration of yeast will protect rats against the appearance of hepatic cirrhosis produced by p-dimethyl-amino-azo-benzol (7). Von Glahn and Flinn found that yeast would partially protect the liver of rabbits against the effect of feeding lead arsenate (8). Patek and Post (9, 10) obtained beneficial effects in cases of human cirrhosis by feeding a nutritious diet supplemented by vitamin B concentrates, but could not find any protective effect of yeast on experimentally produced carbon tetrachloride cirrhosis in rats (11). They found however, that rats receiving 8 to 11 grams of food per day had more severe hepatic lesions than rats fed 14 grams per day. In our experiments on hyperthyroid dogs the animals receiving the high B vitamin diet, with which it took an average of 90 days to produce an abnormal liver function, ate approximately 1.5 times as much food as normal. This increased voluntary food intake is necessary (4, 17), and was sufficient, to prevent a loss of weight in the hyperthyroid dogs. Thus, this increased food intake is a possible factor in delaying the appearance of an abnormal liver function. If this is so, it is still necessary that the high B vitamin diet be supplied if the food intake is to be increased.

Pulse rate. As previously noted (2, 12), the cardiac symptoms of beri-beri and hyperthyroidism in humans are quite similar. In man tachycardia is produced in both diseases. Thus a deficiency of thiamine would be expected to intensify any tachycardia produced by Graves' disease. However, in the dog a

deficiency of thiamine tends to produce bradycardia. When the yeast is removed from the diet of a hyperthyroid dog the pulse rate rapidly falls to normal and can be raised to its hyperthyroid level by an injection of thiamine (2). In Graves' disease the administration of thiamine should tend to reduce the pulse rate if any degree of a thiamine deficiency previously existed. Frazier and Ravdin (12) have observed a reduction in the pulse rate of hyperthyroid patients treated with thiamine and yeast. The studies in this paper have shown that when the dietary yeast is removed at the time thyroid feeding is started, only a slight tachycardia is obtained which returns to normal within 10 days. This again shows that production of tachycardia in thyroid-fed dogs depends on an adequate supply of vitamin B_1. If thiamine and yeast are administered to hyperthyroid dogs who have had the yeast removed from their diet the pulse rate will rise to the previous hyperthyroid level, but despite this treatment the pulse rate will return towards normal if thyroid feeding is continued for 80 to 100 days. Likewise, the pulse rate of hyperthyroid dogs receiving a high B vitamin from the start of thyroid feeding also begins to return to normal after a similar period of thyroid administration. The fall in pulse rate at this time is associated with a hypertrophy of the heart (table 2).

The evidence in the literature demonstrates that the B vitamins play an important rôle in the symptoms and changes produced by feeding thyroid gland (1–4, 13). There is also some clinical evidence to support this view (12, 14, 15). It should be emphasized that the B vitamins will not counteract *the effects of hyperthyroidism per se, either experimental or clinical, but will* only effect such symptoms as are produced by a deficiency of these B vitamins, the deficiency of the B vitamins, or an increased requirement for, being in turn produced by thyroid feeding (16, 17).

CONCLUSIONS

1. A yeast-free diet renders dogs extremely susceptible to the effects of thyroid feeding, an abnormal liver function being produced in an average of 22 days. Thyroid-fed dogs receiving a small amount of yeast each day developed an abnormal liver function in an average of 45 days. When hyperthyroid dogs were fed a high B vitamin diet it took an average of 90 days before an abnormal liver function was observed. Thus, the amount of B vitamins fed has a direct relationship to the time elapsed before an abnormal liver function develops. A high B vitamin diet will delay but will not prevent the appearance of an abnormal liver function in hyperthyroid dogs.

2. Hyperthyroid dogs fed a yeast-free diet develop only a slight tachycardia which returns to normal within 10 days. Thus, in an absence of thiamine (vitamin B_1) thyroid feeding does not produce tachycardia.

3. *Hyperthyroid dogs receiving a high B vitamin* diet maintained a high pulse rate for about 80 to 100 days of thyroid feeding, after which time the pulse rate fell toward normal. The drop in pulse rate at this time seemed to be correlated with a compensatory hypertrophy of the heart.

4. The relation of these changes to those seen in Graves' disease is discussed.

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EFFECT OF ULTRAVIOLET RADIATION ON BODY WEIGHT OF MICE

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The following observations on body weight were made in the course of investigations of the carcinogenic action of ultraviolet radiation. Large numbers of animals were involved in these studies; in fact more than might justifiably be devoted to an investigation dealing solely with the present topic. Hence the measurements obtained are highly dependable, but on the other hand the experimental attack has not been as direct as had this been an independent study.

EFFECT OF MERCURY ARC RADIATION. *Method.* The method of dosage has been discussed elsewhere (Blum, Kirby-Smith and Grady, 1941), and only a brief description will be necessary here. The source of radiation was an intermediate pressure mercury arc enclosed in quartz. The amount of radiation of wavelengths shorter than 3200A[1] emitted by this lamp was measured continuously throughout the period of exposure by means of a titanium photocell and integrating recorder. Thus, dosage and intensity of radiation were accurately controlled.

Two to three months old inbred, albino mice (strain A) were used. They were given a diet of Purina dog chow *ad libitum* except in one experiment where diet was purposely restricted. Each experimental group was made up of 26 animals at the beginning. Average body weights were obtained from weekly group weighings. The standard deviation for individual body weights, determined for several of the groups was approximately 10 per cent of the group mean. At higher doses some of the animals developed tumors after about 110 days and were killed for study; hence the average body weights are less reliable after this time.

Dose-body weight relationship. Figure 1 shows average body weights of several groups of mice each receiving five exposures to ultraviolet radiation per week. A relative decrease in average body weight of exposed as compared with control animals is clearly shown, this effect being greater at higher dosages. Lower body weight for animals exposed to mercury arc radiation as compared to control animals was reported by Ellinger (1938, 1939) whose observation is amply confirmed by these findings.

The relationship between body weight and dosage is illustrated in figure 2. For comparison, four of the weekly weighings have been averaged, namely, those of the 16th to 19th weeks after irradiation began. This is a good period for comparison since the group body weight becomes approximately constant at this time, yet the incidence of tumors is still low. The essential relationship

[1] See Blum, Kirby-Smith, and Grady (1941) for the spectral relationships involved. The importance of this wavelength limit will become apparent in later discussion (p. 380).

is not altered if another period is chosen for the comparison. Some of the points in the figure were obtained by averaging weights of two or more groups that received the same dose.

The relationship between body weight and logarithm of dose is linear down to a certain dosage below which body weight does not vary with dose; this value is somewhat different for each experiment. In experiments II and IV (fig. 2) the broken lines represent body weights of control groups. In experiments I and III no controls were maintained, but the constancy of body weight below a given dosage clearly indicates this as the control weight. The differences in body weight among the control and low dosage groups probably represent group and seasonal differences. Experiments I and II were practically concurrent whereas III and IV were carried out at another season. All the data indicate

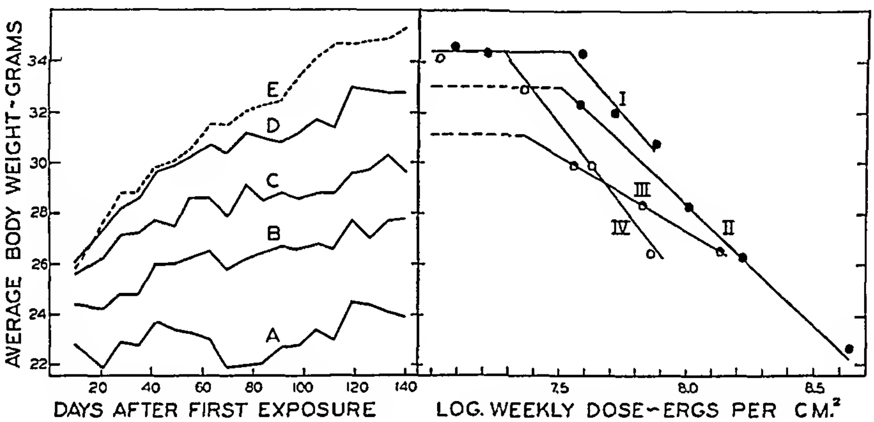

Fig. 1 Fig. 2

Fig. 1. Average body weights of mice exposed to different dosages of radiation from an intermediate pressure mercury arc. Weekly doses as follows: A, 43.0 × 10^7; B, 16.5 × 10^7; C, 9.9 × 10^7; D, 3.6 × 10^7 ergs per cm² of wavelengths shorter than 3200A, E, control. The weekly dose was given in this experiment in equal exposures on five days of the week.

Fig. 2. Relationships between body weight and dosage of radiation. In experiments I and II the weekly dose was given in five equal exposures per week; in III in seven equal exposures, and in IV in a single weekly exposure. The broken lines represent control weights.

that doses below a critical value do not affect body weight. There is no evidence that increase in body weight is stimulated by any dosage.

Three different schedules of dosage were used. In experiment IV the total weekly dose was given on one day per week, in experiments I and II it was divided into five equal exposures administered on five days, and in III into seven equal exposures, administered daily. The interval between doses affects the results quantitatively but does not alter the essential relationships. In general, larger doses given at longer intervals are more effective than smaller, more frequent doses. Destruction of tissue is also more marked under the former conditions (see p. 383).

If the exposures are interrupted the average body weight of the group increases rapidly, and may soon reach that of the controls.

Food intake. In one experiment the food consumption of irradiated and control animals was followed daily, and found to be distinctly less for the former. Paired feedings were not carried out, but after the experiment had continued for some weeks the food consumption of the controls was restricted to approximately that of the irradiated animals with the result that the body weight of the controls fell rapidly, almost to that of the irradiated mice. Thus, depression of body weight by ultraviolet radiation appears due to decreased food consumption rather than to qualitative modification of metabolism. The decreased food intake is accompanied by, and may result from, a general decrease in activity of the mice. It was noticeable that fighting among the animals decreased with increase in dosage of radiation, the irradiated mice being quieter and better groomed than the controls.

These facts throw doubt upon the hypothesis of Ellinger (1938, 1939) that the decreased body weight is due to increased thyroid gland activity indirectly stimulated by histamine produced in the skin by ultraviolet radiation. We have observed no gross or microscopic changes in this gland.

Effect of intensity. In some experiments the intensity of the radiation was varied, but constant dosage maintained. When the intensity was varied tenfold no differences in body weight were observed. In two groups each of fifty animals, in which a twenty-fold difference in intensity was maintained, the body weight was 3.5 per cent less for the group receiving the highest intensity. This suggests a slightly lower effectiveness for the lower intensity, but the difference is not statistically significant. Thus, the reciprocity law (Intensity $\times$ time = a constant) must hold within rather wide limits.

The effect of confinement of the animals in small cages during the period of exposure is shown by this observation to be unimportant since those groups receiving low intensities were confined much longer than those receiving high intensities.

THE ACTION SPECTRUM. In certain experiments the wavelength range was restricted by interposing Corex D, Pyrex, or window glass filters between the source and the animals. The window glass filter cuts off virtually all energy of wavelength 3200A and shorter. Mercury arc radiation passing through this filter does not affect body weight whereas that passing the Pyrex filter, which is confined principally to the 3130A line, does. Hence the long wavelength limit for this effect must lie between the adjacent 3130A and 3340A lines of the mercury arc. For this reason, wavelengths longer than 3130A are disregarded in the following tables.

Table 1 shows the intensities of the lines of the mercury arc incident upon the mice when no filter is interposed. These are expressed in ergs per cm.^{2} per second per line. The dosage, expressed in ergs per cm.^{2} is obtained by multiplying the intensity by the number of seconds of exposure. The transmissions of these lines by the Corex D and Pyrex filters are also given in this table. For a given line, the product of the intensity and the transmission gives the intensity incident on the mice when the particular filter is used. The sum of these values for all the lines, divided by the sum of the intensities of all the lines when no filter is

interposed, multiplied by the number of seconds of exposure gives the dosage incident through the filter.[2]

An experiment was also carried out with a low pressure mercury arc, which emits over 90 per cent of its energy as wavelength 2537A. About four per cent of the remainder is in the 3130A, 3020A and 2967A lines, but any effect of these was compensated by exposing the control for this group to the radiation from this source passing through a Corex D filter. By this arrangement the control did not receive wavelength 2537A, but virtually all the radiation of other wavelengths.

Table 2 summarizes the results of these experiments. Due as much to good fortune as design, the same reduction in body weight from that of the controls (6 per cent) was observed with the Pyrex and Corex D filters and with the 2537A line. For comparison, the dose without filter which would bring about the same per cent reduction in body weight was estimated by interpolation from data obtained from a number of experiments. Thus the fourth column of the table presents the amounts of energy of various wavelength regions required to bring about a six per cent reduction in body weight.[3]

TABLE 1

Spectral data

WAVE-LENGTH λ	INCIDENT ENERGY WITHOUT FILTER (<λ3200A)	FRACTION OF INCIDENT ENERGY TRANSMITTED BY:		
		Corex D filter	Pyrex filter	Irradiated-epidermis*
	ergs cm^{-2} sec^{-1} × 10^{3}			
3130A	13.2	0.76	0.40	0.28
3022A	6.8	0.64	0.12	0.23
2967A	3.8	0.52	0.08	0.17
2925A	0.5	0.45		0.14
2894A	1.2	0.40		0.08
2804A	2.4	0.17		0.02
2752A	0.8	0.10		0.02
2699A	0.8	0.05		0.02
2652A	4.8	0.02		0.02
2602A	0.2			0.02
2537A	5.3			0.02

* The method for obtaining these values is described by Kirby-Smith, Blum and Grady (1942).

TABLE 2

SOURCE	FILTER	WAVE-LENGTHS	WEEKLY DOSE (WAVE-LENGTHS <3200A)		REDUCTION IN BODY WEIGHT BELOW THAT OF CONTROLS
			Incident on surface	Incident beneath epidermis	
			ergs cm^{-2} × 10^{7}	*ergs cm^{-2} × 10^{7}*	*per cent*
Intermediate pressure mercury arc	Window glass	>3130A	0	0	0
	Pyrex	>2967A	8.0	2.1	6
	Corex D	>2652A	4.1	1.0	6
	None	>2400A	5.3	0.8	6
Low pressure mercury arc	None	2537A	36.0	0.7	6

Whatever the inherent inaccuracies of these measurements, it is obvious that there is a great difference in the effectiveness of different wavelengths, when

[2] Actually the total energy rather than the time of exposure was measured in these experiments (Blum, Kirby-Smith and Grady 1941), but the essential principle of the calculation is the same.

[3] In making such a comparison it should be kept in mind that reduction in body weight varies as the logarithm of the dose.

considered in terms of energy incident on the skin surface. Wavelength 2537A is weakly effective, over four times as much energy being required to achieve the same effect as for wavelength 3100A (Pyrex filter), and nine times as much as for the radiation passing through the Corex D filter.

If changes in living cells are assumed to underlie this phenomenon, the dead corneum of the epidermis must be regarded as a screen which protects the underlying viable layers. The corneum displays selective absorption and must modify the relative effectiveness of the various wavelengths. This is an important factor in determining the character of the action spectrum for the erythema of sunburn of man, which is an analogous process.

An estimate of the magnitude of this effect can be made from measurements of the transmission of the epidermis, since the corneum is responsible for most of the absorption by this layer. Table 1 gives transmission values for the epidermis of the ear of an albino mouse, which had been subjected to about the same amount of radiation as those here compared, and these values have been used in calculating the energies incident beneath the epidermis which appear in column five of table 2. The different wavelengths seem to have nearly the same effectiveness when considered in terms of energy reaching the epidermis.

While these data do not provide an accurate action spectrum, they permit the conclusion that the corneum is not involved in the photochemical processes leading to the reduction of body weight. This helps to rule out the possibility that vitamin D plays a rôle in this phenomenon, since this substance must be formed chiefly in the superficial layer (see Blum, 1942). Wavelength 3130A is almost without effect in the production of vitamin D, whereas 2537A is quite effective (Bunker and Harris, 1937; Knudson and Benford, 1938), and this is the reverse of the relative effectiveness of these wavelengths in reducing body weight. This accords with Blum and Lippincott's (1942) failure to find evidence of hypervitaminosis D among these mice.

The long wavelength limit at 3200A places the action spectrum in that spectral range which produces damage to cells in general, among which effects the changes underlying the erythema of sunburn of man may be included. It is probable that protein (see Mitchell, 1938; Blum, 1941) is the substance primarily affected in this destructive reaction. Nucleic acid seems the only alternative substance, and in either case the long wavelength limit would have to be approximately that found experimentally. The falling off of the absorption spectra of these substances toward the long wavelength limit could account for the lower effectiveness of wavelength 3130A.

In recent years it has been shown that light influences the sexual cycle of certain mammals, and hence might affect metabolism and body weight. Such effects are brought about by wavelengths longer than 3200A (approximately those of the visible spectrum), however, and could not be responsible for the reduction of body weight found in the present experiments.

The effects of longer wavelengths on growth found by Luce-Clausen and Brown (1939) could not play a significant rôle under the experimental conditions we have used.

PATHOLOGICAL FINDINGS. Grossly, such radiation causes changes in the parts not covered by hair, namely, the ears, tails, paws, snouts, eyelids and eyes. The hair itself may display a yellow color after severe dosage, but the skin beneath the hair is little affected if at all. The ears show the greatest change, and have been subjected to the most thorough histological studies because they are the site of the great majority of tumors (Grady, Blum and Kirby-Smith 1941). Prior to the appearance of tumors, i.e., during the period body weight comparisons were made, the ears show changes varying with the dose from the mildest hyperplasia of the epidermis and deeper tissues to severe inflammation and necrosis. The magnitude of the change in body weight parallels the amounts of damage to the skin of the ears.

No pathological changes in internal organs, either gross or miscroscopic, were found which could be correlated with the external changes or with body weight effects. No abnormalities of the thyroid glands were observed.

DISCUSSION. It seems clearly established that ultraviolet radiation of wavelength shorter than 3200A slows the normal gain, and may even cause a decrease in body weight of mice. This is directly traceable to decreased food consumption, and parallels damage to cells of the skin. The interrelations of these events are obscure. While the hypothesis that toxic products (possibly histamine-like substances as suggested by Ellinger (1938, 1939)) resulting from cell damage underlie the decreased food intake seems most reasonable, direct proof is lacking and systemic changes that might be expected to result, have not been found. Other explanations might be offered, but failing supporting evidence prolonged discussion is not justified. Qualitative changes in metabolism are not demonstrated, and the absence of evidence of increase in body weight at any dosage is striking when one considers that benefits to general health are so commonly claimed for exposure to ultraviolet radiation. Caution must be exercised in applying these findings to man, however, for direct quantitative comparison cannot be made because of differences in transmission characteristics of the epidermis.

SUMMARY

Ultraviolet radiation of wavelengths shorter than 3200A slows normal gain or may reduce the body weight of mice. Below a certain dosage body weight is not affected. There is no evidence of growth stimulation at any dosage.

The extent to which body weight is affected varies with the total dosage and interval between doses, but within the limits explored intensity of the radiation is not a factor.

The lowering of body weight seems due to decreased food consumption.

Wavelength studies indicate that the radiation directly affects the living tissues of the skin, the corneum acting as a semi-opaque screen which protects the deeper layers.

The lowered body weight parallels destructive changes in the skin, but evidence of important changes in internal organs is lacking.

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THE EFFECTS OF VITAMIN D AND OTHER STEROLS ON BLOOD PRESSURE IN THE RAT[1]

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The earlier literature on toxic responses to vitamin D_2, has been reviewed repeatedly (1, 2, 3, 4). The effects on the kidney and on the vascular system have received considerable attention. Appelrot (5) reported that toxic doses of vitamin D_2 produced hypertension in dogs and suggested similarity between this action and that of adrenalin. He believed the hypertension to be due to hypertrophy of the media of the arterioles.

Goormaghtigh and Handovsky (6) also have found hypertension in dogs, with hypertrophy of medial muscle fibers in the arterioles of the kidneys, spleen and intestines, often followed by atrophy. Also, there was increased sensitivity to adrenalin.

The hypertrophic response to vitamin D_2 affected not only the smooth muscle cells, but also the afibrillar cells and the paucifibrillar cells as well. Very large doses, on the other hand produced necrotic degeneration and hypotension.

Grollman, Harrison and Williams (7) produced hypertension in rats with a number of other sterols, such as desoxycorticosterone, diethylstilbestrol and estradiol benzoate.

It is the purpose of this paper to present the results of observations on blood pressure in rats receiving toxic doses of vitamin D_2. After some preliminary consideration of methods, that of Byrom and Wilson (8) was selected as most suitable with some minor modifications, as follows:

The rat was anesthetized lightly in a battery jar, the top of which was fitted with an ether-soaked cotton pad. The sedated rat was placed on a platform and the tail inserted through a specially constructed cuff modified from that used by Williams, Harrison and Grollman (9). A glass tube filled with water served as a plethysmograph into which the distal extension of the tail was inserted. The proximal end of the plethysmograph was sealed around the tail with vaseline.

The distal end of the plethysmograph was connected with a capillary tube. The meniscus of the water column was adjusted in the capillary tube by a screw plunger in a side arm of the plethysmograph.

An ordinary clinical mercury manometer was attached to the compression cuff but with a T-tube inserted for attachment of the pressure bulb. With the tube clamped between this and the cuff the pressure was raised to 200 mm. Hg. The clamp was then released and the entire pressure suddenly applied so as to shut off

[1] A part of the expenses of this investigation was borne by a grant from the Nutrition Research Laboratories.

[2] Submitted in partial fulfillment of requirements for the degree of Master of Science, 1941.

all arterial flow into the peripheral tail. The pressure was then lowered gradually with the bulb release valve. When the systolic pressure was reached in the compression cuff, there was immediate filling of the tail vessels. The consequent increase in volume caused a sharp movement of the water meniscus in the capillary tube. The manometer was read at this point. Several readings could be made on the same animal before recovery from anesthesia.

Experimental procedure. Albino rats of both sexes, selected from our own homogeneous colony, were used, with litter mates as controls wherever possible. All of the animals were maintained on a diet of Fox Chow pellets (a commercial preparation) and of tap water ad libitum. They also received weekly rations of lettuce, carrots and whole milk. The experimental rats were fed activated ergosterol[3] dissolved in oil. This was administered by stomach tube, to which the animals very quickly became accustomed. The control rats were fed duplicate volumes of corn oil. Each animal was weighed every 2 to 3 days, and the blood pressure was taken every 3 to 5 days by the technique described. Blood pressure determinations at shorter intervals were deemed inadvisable because of the possible adverse effects of too-frequent ether anesthesia.

Group I comprised twenty rats. After prliminary training and standardization of blood pressure, each animal received a daily dose of 300 units of vitamin D_2 per gram of body weight over a period of 4 days. Toxicity, as manifested by weight loss (upper graphs, fig. 1), was so pronounced that a recovery period was allowed, after which administration was resumed at a daily dose of 30 units per gram as shown in figure 1.

Since this dosage appeared to be innocuous the daily dose was increased to 150 units per gram for 5 days, when weight loss again indicated toxicity. The records of 5 animals, selected at random, were graphed for figure 1. From February 28 to June 15, no observations were made. During the next two weeks a total of 53 readings was made in the group with a mean value of 105 mm. Hg, as compared to a mean of 110 mm. when the last previous reading was made.

With the exception of no. 8, none of the animals in this group showed any significant increase in blood pressure, the mean maximum being 137 mm. Hg at the termination of vitamin D_2 feeding. From figure 1, it will be seen that this hypertension developed during a period of toxication with pronounced weight loss. In *all* other instances weight loss was accompanied by hypotension. Three months later the blood pressure was *98 mm. Hg*. It seems probable, therefore, that this was a true hypertension.

Control group. Eleven litter mate controls were observed for nearly 8 weeks then given volumes of corn oil identical with those in which the vitamin D_2 was given. There was a tendency to progressive increase in the blood pressure almost directly parallel with the increase in body weight. With corn oil feeding for 4 weeks there was a slight tendency to decreased pressure, but this was not marked. After three months the mean of 61 readings was 106 mm., slightly lower than the mean maximum of 117. Due to hot weather the mean weight for the group had decreased also.

[3] Supplied by Abbott Laboratories and Winthrop Chemical Company.

Group II comprised 4 rats (fig. 2). These animals received daily 150 units of vitamin D_2 per gram for 5 days. With prompt weight loss, severe hypotension developed in three animals. Recovery of normotension began with resumption of weight gain 2 or 3 weeks later. Number 19 did not show a very severe weight loss and the hypotension was less severe than in the others. The mean of 8 readings taken three months later was still only 100, although the mean weight was 414 grams, 90 grams more than the previous maximum.

Group III comprised 11 rats that received, daily, 75 units of vitamin D_2 per gram for 56 days. Another control group of like number received identical volumes of corn oil daily for this period. The mean weight gains were identical. It cannot be said that the slight differences in blood pressure readings are significant, although the vitamin group did show a slightly greater mean maximum.

After three months the mean of 45 readings in the vitamin group was 110 mm. Hg as compared to the last previous reading of 124.

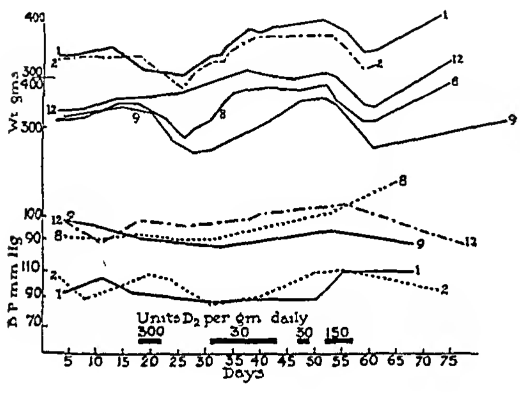

Fig. 1

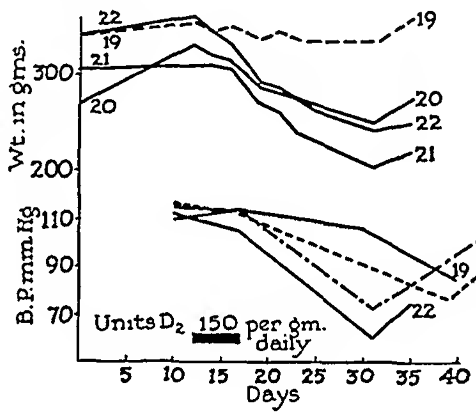

Fig. 2

Fig. 1. Body weight and blood pressure in 5 rats of group 1, receiving vitamin D_2 shown.
Fig. 2. Body weight and blood pressure of 4 rats selected at random from group 2.

Group IV. In order to test the accuracy of the method 6 male rats each received a daily intramuscular injection of 0.5 mgm. of desoxycorticosterone[4] over 10 days. This substance has been proven to be hypertensive for rats, so is regarded as a satisfactory test substance for this purpose. Weight changes during the period were not pronounced, but the mean blood pressure rose from 124 mm. Hg to a maximum of 147 mm. two days after the last injection. Ten days later, hypertension of 135 mm. still persisted. Three months later the mean of 18 readings in this group was 115 mm.

Discussion. The results obtained with desoxycorticosterone prove the method a valid one for studying hypertension in rats. The failure to produce hypertension with daily doses of 150 to 300 units of vitamin D_2 per gram of body weight might be explained as due to the extreme degree of toxicity as manifested by weight loss. However, it might also be expected that such an episode might produce a delayed hypertension. Other studies would lead one to predict this

[4] Supplied by Ciba Company.

result, at any rate, since many of the metabolic disturbances seen in vitamin D_2 toxication persist for 6 to 8 months.

The two groups of observations on corn oil feeding prove that is not a factor of importance in any of the results observed in this study.

The results of administration of vitamin D_2 on the basis of body weight show that daily doses up to 75 units per gram may be tolerated indefinitely without hypertension or weight loss and without any delayed effects. It is in these dosage ranges that one might expect delayed chronic hypertension to develop. This, however, did not occur. In the delayed observations no hypertension was found.

Somewhere between 75 and 150 units per gram (75,000–150,000 per kilo) lies the toxic threshold, where weight loss and hypotension are found. The rat is, therefore, about 5 to 6 times as resistant to vitamin D_2 toxication as the dog and human. According to the recent report of McChesney and Kocher (10), doses of about one-fifth this magnitude are capable of producing hypercalcemia in rats. It appears, therefore, that the hypercalcemic and toxic effects are not closely correlated.

The hypertension that has been reported as occurring in dogs has been explained variously. One explanation is that the vitamin produces arteriosclerosis in the renal arteries, and that the ischemia resulting brings on chronic renin hypertension. Another is that the vitamin produces hypertrophy of the afibrillar or paucifibrillar cells in the preglomerular arterioles. These cells have been charged with responsibility for an endocrine function, i.e., production of a vasopressor substance which, if present in sufficient concentration, would produce vascular spasm and chronic renal ischemia.

Since there have been reports on differences in toxicity among different forms of vitamin D, it now seemed advisable to determine whether other preparations might be more effective in producing hypertension.

The following experiments were designed to test two other products, vitamin D_3 [5] and Ertron.[6]

Two groups of albino rats, of the same stock as those used in the earlier work, were used. Each set consisted of two sub-groups, male and female. Each rat served as its own control for a period of 30 to 40 days before the vitamin was introduced.

Group V, comprising 5 females and 4 males, during a control period ranged between 90 and 115 mm. Hg in blood pressure and maintained a steady weight. Vitamin D_3 then was given in a daily dose of 75 units per gram of body weight until death resulted from toxicity. The mean values for the blood pressures and weights for both groups showed a parallel decline. The average values for the males declined from 92 mm. Hg and 308 grams weight to 70 mm. Hg and 235 grams. The final rise (fig. 2) reflects the influence of a last surviving rat. The mean values for the females declined from 95 and 220 to 87 mm. Hg and 151 grams. In no case was hypertension seen.

[5] Courtesy of Dr. J. Waddell of Du Pont Co.

[6] Courtesy of Nutrition Research Laboratories.

Group VI, 4 females and 6 males, averaged between 90 and 120 mm. Hg pressure and maintained a steady weight during the control period. Ertron then was given in a daily dose of 75 units per gram of body weight until death resulted from toxicity. The male group showed a decrease in blood pressure and weight. The female group showed a decline in weight, but there was no significant change in blood pressure. The rise at the end of the experimental period (fig. 3) again was due to a single survivor. In no case was hypertension observed. The results obtained with these products show that neither causes hypertension in rats.

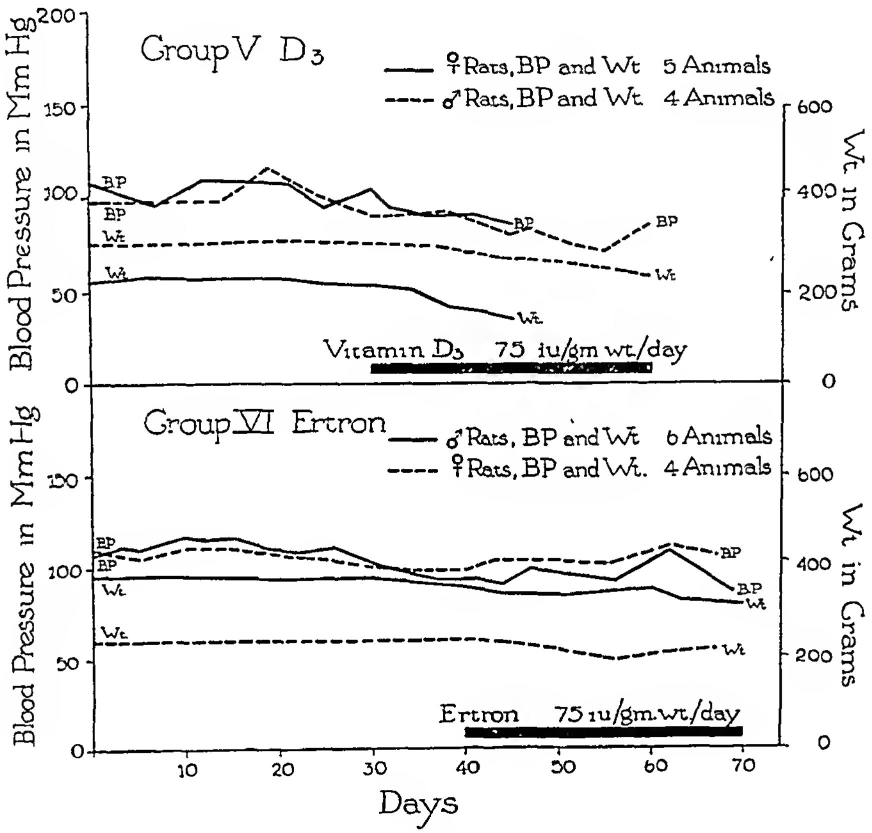

Fig. 3. Mean weight and blood pressure of rats receiving vitamin D_3 and ertron. Groups 5 and 6.

These experiments on rats do not confirm the findings of hypertension in dogs after heavy administration of vitamin D_2 reported by others. The reason for the discrepancy is not apparent at present. Frequent attempts have been made in this laboratory to demonstrate in both dogs and humans the production of hypertension by means of various forms of vitamin D, but without success. Further efforts are being made to induce hypertension in dogs.

CONCLUSIONS

1. Blood pressure measured in the tail of the albino rat is not increased, either acutely or chronically, by the oral administration of vitamin D_2 in doses sufficiently large to induce severe toxication as manifested by a marked loss of weight.

2. In growing, untreated, control animals, there is a small increase in blood pressure that is approximately parallel with the weight gain.

3. With loss of weight from toxication the blood pressure tends to be decreased concomitantly with the weight loss.

4. Desoxycorticosterone produces marked hypertension, as reported by others.

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THE RÔLE OF PRESSORECEPTORS IN THE REGULATION OF BLOOD PRESSURE IN THE RABBIT

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Recent work on regulatory mechanisms for the circulation have shown that important sources of afferent impulses are located in the carotid sinus, the arch of the aorta and the Pacinian corpuscles of the mesentery. It was confirmed by Heymans *et al.* in 1936 that, in the absence of the sensitive zones in the carotid sinus and aortic arch, changes in general blood pressure still cause compensatory reactions in vascular tone. They suggested that the clue to these reactions might be found in the mesenteric area. Gammon and Bronk (1935) had already noted that the Pacinian corpuscles of the mesenteric area signal the degree of distention of the mesenteric vessels by afferent impulses sent along the splanchnic nerves. Since no observations had been made on the rabbit on the rôle of the splanchnic area in connection with other reflexogenic zones, it was decided to study the effects of elimination of these sources of afferent impulses during tipping experiments in the foot-down position (hind feet down, head up), where compensation for the action of gravity might be expected.

METHOD. The rabbits were anesthetized with urethane, with the addition of some ether during the splanchnic isolation. Blood pressure was recorded with a modified Hürthle manometer from a cannula in the left carotid artery. The average blood pressure was determined by measuring a large number of systolic and diastolic pressures. This method was used in preference to the more laborious one of calculating mean pressures by the planimeter, since a comparison of the two methods showed them to be closely in accord. This had been observed by Edholm also in 1940. In five experiments records of respiration were included, using a pneumograph and tambour.

A special animal board was used for the tipping, arranged so that the angle of rotation was just caudal to the rabbit's heart. Animals were tipped for 20 seconds, in the earlier experiments to 30°, 45° and 60°. Later, as the rabbit's ability to compensate became more evident, the lower degrees of tipping were omitted and only 60° and 75° were used. Only the experiments at 60° and 75° are reported here. To prevent sliding in the upright position the rabbit's hind feet were braced and lightly tied against a stop at the lower end of the board.

Two semi-cylindrical, adjustable metal uprights were used under the axillae. The rubber tubing connected directly with the carotid cannula was encased in a metal collar clamped to the animal board, and held firmly in place by sewing it to the rabbit's skin.

The order in which the nerves were cut was varied, starting sometimes with the vagi and aortic depressors and sometimes with the splanchnics. The vagi in the rabbit contain some depressor fibers, so it was necessary to cut them as well as the separate aortic nerves. The use of the left carotid artery for blood pressure registration eliminated the left carotid sinus. The right carotid sinus was eliminated temporarily during a tipping experiment by clamping the right carotid artery. Clamping had already been compared with denervation (Conklin and Dewey, 1941), as to its effect on the reflexes under consideration here, and had been found to give identical results. The splanchnic nerves were isolated by the extra-peritoneal approach, in all but two cases. Ligatures were laid under them and they were eventually severed by a sharp pull on the ligatures, without changing the position of the animal. At autopsy in each rabbit there was careful verification of all nerves severed. This was especially necessary in the case of the splanchnics, where the number of strands, often obscured by fat, varies from one to five on a single side, and where two sides are seldom alike. No experiment was included in the results where any splanchnic twig was left unsevered.

Results. The responses of normal animals were, in the great majority of cases, as follows: the blood pressure dropped rapidly with the tipping, but showed a marked compensatory rise while the animal was still tipped; a few animals, notably those with low blood pressure to begin with, did not show a compensatory rise, or even had a slight continuous fall, but in general the response was positive and vigorous. Quite often an intermediate rise occurred at about 10 seconds. The characteristic reaction is shown in figure 1, A. The drop in the blood pressure during the tipping period may be seen, then the compensatory rise and the overshoot at the end, when the animal was returned to the horizontal position. This overshoot was nearly always present and was thought to be evidence of increased stroke volume of the heart due to the hydrostatic effect.

When the vagi and aortic nerves were cut the rabbit made the same compensatory response to tipping (fig. 1, B) except that in some cases the fall of pressure was *less* and the recovery *more* complete, undoubtedly due to the removal of the inhibitory vagus impulses. Respiratory waves are prominent in such records.

When, in addition to cutting the vagi and aortic nerves the right carotid artery was temporarily clamped, so that both carotid sinuses were thrown out of commission, there was an immediate increase in the level of the blood pressure before tipping, a smaller fall when tipped, a better compensation, and often a recovery in the horizontal position to a higher pressure than the initial one (fig. 1, C). So far, the experiments corroborate those of Koch (1935) and Conklin and Dewey (1941).

When, following the elimination of the buffer nerves and the carotid sinuses, the splanchnics were cut, there was, of course, a lowering of general blood pres-

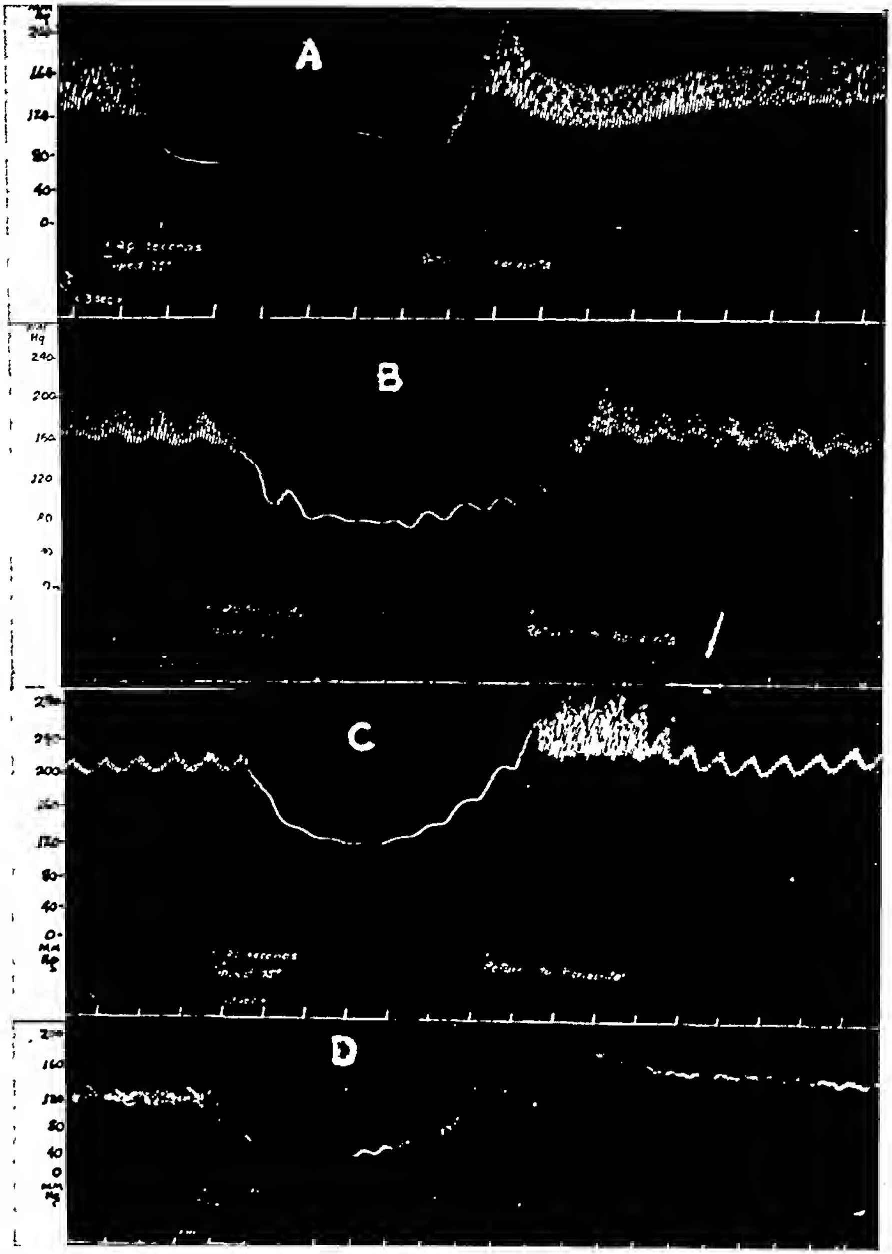

Fig. 1. Blood pressure response to 75° tipping, foot-down position, with progressive elimination of receptor areas. A: normal (cannula in carotid artery). B: vagus and aortic nerves cut. C: vagus and aortic nerves cut, second carotid artery clamped. D: vagus, aortic and splanchnic nerves cut, second carotid artery clamped. Upper line: blood pressure. Middle line: tipping signals. Lowest line: 3-second time signals.

sure, but again a compensatory rise during the tipping period (fig. 1, D). When the splanchnic nerves were cut first, followed by cutting of the buffer nerves

and clamping of the right carotid artery, the effect of splanchnic vasodilatation was rather more marked. There were a few cases where the blood pressure remained the same during the period of tipping. The majority showed a rise, though not a marked one. A rise was also shown in records taken after the splanchnics only had been cut.

Table 1 is a summary of all the experiments at 60° and 75° tipping. It shows the compensatory rise of blood pressure in millimeters Hg during tipping from the time of the lowest pressures reached to the end of 20 seconds, just before the return to the horizontal position. In the experiments with 60° tipping it will be seen that: 1. Elimination of vagus impulses has a favorable effect on compensation. 2. Cutting out both carotid sinuses after vagus section does not disturb compensation, but may even improve it slightly. 3. With elimination of the splanchnics alone, the compensation is lessened, due to splanchnic vasodilatation.

TABLE 1

Mean blood pressure gains during tipping

PROCEDURE (IN ORDER)	60°			75°		
	No. of expts.	Range	Av.	No. of expts.	Range	Av.
		mm. Hg	*mm. Hg*		*mm. Hg*	*mm. Hg*
Normal	8	+8 to +48	+26.5	8	+18 to +62	+34.9
Vagi and aortic nerves cut	8	+26 to +67	+42.0			
Vagi, aortic nerves cut; carotid artery clamped	5	0 to +60	+28.0			
Vagi, aortic nerves cut; carotid artery clamped; splanchnic nerves cut	3	0 to +36	+20.0	2	0 to +28	+14.0
Splanchnic, vagi and aortic nerves cut; carotid artery clamped	8	−2 to +52	+12.0	5	−13 to +35	+10.0
Splanchnic nerves only cut	9	0 to +26	+7.1	6	0 to +27	+13.5

4. Elimination of the buffer nerves, carotid sinuses and splanchnics, whether the splanchnics are cut first or last, still leaves compensatory reactions at work. The experiments at 75°, though not so numerous, show an equivalent response.

In three cases longer tippings at 75° were done on rabbits deprived of buffer nerves, carotid sinuses and splanchnic nerves. A slow rise in pressure occurred in these animals while they remained tipped. One made a gain of 105 mm. in 3 minutes; a second made a gain of 35 mm. in 6 minutes; and a third made a gain of 37 mm. in 9 minutes. These experiments indicate that our 20 second tipping period was too short in some cases to bring out the full capacity of these animals for compensation.

DISCUSSION. The decrease in arterial blood pressure when an individual is tipped to the vertical or near vertical position has been measured in man and several animals. A compensatory rise has been observed in man during the tipping period by many investigators; in cats by Edholm (1940); sometimes in

dogs by Wald, Guernsey and Seott (1937) and Mayerson (1941); and in rabbits by Koeh (1935).

It was expeeted that the rabbit would be even more sensitive than other species to the elimination of the usual sourees of afferent impulses. This was found not to be so. It has been shown that the rabbit retains a eonsiderable power of eompensation even when all well-known sourees of afferent impulses are eut off.

In the eases where respiration was recorded it never failed during the tipping experiments, but was either maintained at the same rate during tipping or at a somewhat redueed rate, if the initial rate had been very high. The shortness of the period during whieh the animal was tipped and the maintenanee of respiration in all cases during this period are indireet evidence against anoxia.

SUMMARY

Brief tipping experiments have been done on 32 rabbits with progressive elimination of sources of afferent impulses causing reflex cireulatory compensation. It has been shown that the rabbit is still able to compensate to some extent for the effect of gravity when it is deprived of vagi, aortic nerves, carotid sinuses and splanehnie nerves. Some other reflexogenic sources, responsible for the compensation, must exist.

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GLUCONEOGENESIS AND CELLULAR INJURY. A FURTHER INQUIRY INTO THE MECHANISM INVOLVED IN DIABETES ENHANCED BY INFLAMMATION[1,2]

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It is well known that the course of diabetes is as a rule accentuated by superimposed infection or inflammation. Recent work has indicated that the mechanism responsible for this enhanced condition seems to be primarily referable to an increased degree of proteolysis at the site of inflammation (1). Gluconeogenesis from such protein breakdown products occurs in the inflamed area (1). The *excess glucose formed locally in turn diffuses into the circulation, thus giving* rise to an excessive hyperglycemia (1). These studies have revealed a higher glucose concentration in the exudate of the inflamed area than in the blood stream, thus supporting strongly other observed facts that the bulk of the sugar is formed at the site of injury rather than originating from the blood.

The earlier literature on the subject has been adequately reviewed elsewhere (1). The present studies represent further data to substantiate the above concept. In brief, glucose apparently seems to be formed in an acutely inflamed area of a non-diabetic dog, but this effect appears to be transitory, for it is soon masked by the developing glycolysis occurring at the site of inflammation. On the other hand, in the diabetic animal the formation of sugar from proteins in an inflamed area is considerably more pronounced and sustained so that it even transcends the elevated level of glycolysis. The result is marked local glucose formation with subsequent diffusion into the circulating blood.

EXPERIMENTAL. The observations were all made on non-diabetic animals and on dogs rendered diabetic by pancreatectomy. The method adopted to withdraw blood samples and exudative material from the pleural cavity of dogs that had previously received an intrapleural injection of about 1.5 cc. of turpentine has been described elsewhere (1). Blood and exudate sugar, total proteins, urea and lactic acid were determined by methods outlined in the earlier study (1). Samples of both exudate and blood were studied at frequent intervals after the introduction of the irritant into the pleural cavity in an endeavor to determine whether a gradient in the concentration of the various substances studied was found to exist between exudate and blood in the earliest stages of the inflammatory reaction. The presence of such a gradient might be of significance in throwing further light on the formation of sugar at the site of an acute inflammation in a diabetic animal.

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RESULTS. I. *Observations on diabetic dogs.* The data on the exudate sugar of diabetic dogs at various stages in the development of the inflammatory reaction are conveniently summarized in table 1 and are shown in chart 1. The levels of blood sugar corresponding to the same intervals are listed alongside. It is clear that in all dogs the exudate sugar is at first at a definitely higher level than the blood sugar. The latter gradually rises reaching conspicuously high concentrations within an interval ranging from several hours to about a day.

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The sugar and urea concentration in exudate and blood of diabetic dogs

DOG NO.	APPROXIMATE DURATION OF INFLAMMATION	EXUDATE SUGAR	BLOOD SUGAR	EXUDATE UREA	BLOOD UREA
	hrs.:mins.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
1	0		263.4		36.3
	6:15	500.3	421.1	54.0	50.5
	25:00	537.9	542.4	159.0	132.0
2	0		259.8		11.0
	2:10	351.9	290.0	42.0	33.0
3	0		260.9		17.0
	1:08	309.4	260.7		11.5
	2:18	288.5	258.6		15.5
	4:38	355.0	281.7	19.5	12.0
	7:13	344.8	239.1	23.0	11.5
	25:26	394.8	306.1	41.0	24.5
	48:13	346.8	303.0	37.0	25.5
Reinjected irritant					
Interval after reinjection of irritant	2:22	434.8	377.4	54.0	42.5*
	19:18	566.7	500.0		
4	*0*		187.8		16.5
	3:36	397.4	264.4	24.0	21.5
	6:08	388.3	283.0	31.0	29.0
	25:03	516.2	421.1	122.5	117.0
Reinjected irritant					
Interval after reinjection of irritant	1:47	625.0	463.0	153.5	142.2

DOG NO.	APPROXIMATE DURATION OF INFLAMMATION	EXUDATE SUGAR	BLOOD SUGAR	EXUDATE UREA	BLOOD UREA
	hrs.:mins.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
5	0		259.8		27.5
	1:10	342.9	263.2	24.0	23.5
	2:41	396.1	259.1		
	4:30	439.6	291.3	37.5	37.5
	23:19	543.5	357.1	115.5	96.5
Reinjected irritant					
Interval after reinjection of irritant	1:13	571.5	386.6	98.5	87.5
	3:43	535.7	438.6	104.5	93.0
	24:02	471.7	490.1	173.0	161.0
(Dalmatian) 6	0		238.1		21.5
	2:00	381.6	242.9		
	5:20	374.0	201.3	37.0	29.5
	24:18	378.6	234.4	24.0	19.5
	46:55	340.5	220.6	22.5	19.5
Reinjected irritant					
Interval after reinjection of irritant	2:02	333.3	211.3	21.5	17.5
	5:25	242.0	195.5	20.5	13.5
	24:34	218.2	209.1	64.5	53.0

* Blood sample withdrawn a half hour later.

The case of dog 6 is an exception. The blood sugar level in this particular animal, with a superimposed pleural inflammation, fails to be enhanced. It is interesting to note that this animal belongs to the Dalmatian breed known to differ in its purine metabolism, i.e., as regards the excretion of uric acid instead of allantoin. Whether this fact bears any relation to the difference in results obtained is not known. It is also to be recalled in this connection that in a large series of diabetic animals several of them may fail to manifest an enhanced blood sugar with superimposed inflammation (2). In general the findings indicate that the blood sugar tends to approach the level of the exudate sugar about a day

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or even at a later period following the introduction of the irritant. Upon reinjection of turpentine in the pleural cavity the concentration of sugar in the exudate is rapidly enhanced to an even higher level. The blood sugar likewise follows such a trend. In brief, the results clearly indicate that with a severe pleural inflammation evidently a gradient due to a lag in the rise in blood sugar is established from the very beginning between the glucose concentration in the exudate and in the blood. This fact suggests that gluconeogenesis occurs at the site of injury. The evidence obtained in control animals, to be presently described, as well as the known inability of glucose to be fixed and thus be concentrated for any appreciable interval in an acutely inflamed area (3) adds further support to the view that the observed gradient can be explained by a process of local gluconeogenesis at the site of inflammation, with gradual diffusion of glucose into the blood stream.

If glucose formation in the inflamed area of a diabetic animal arises from the products of local protein breakdown by deamination, it is reasonable to suppose that some of the constituents of enhanced local protein catabolism might well be recovered in greater concentration at the site of inflammation than in the blood stream. One such product of protein catabolism was therefore studied at length in regard to ascertaining the presence of a gradient between its concentration in the exudate and in the blood stream. This was urea. The data are likewise assembled in table 1. It is clear that in the case of this substance a concentration gradient also exists between exudate and blood. It is, however, considerably less striking than in the case of sugar. This difference may well be referable to the greater diffusibility of urea which would thus tend to be rapidly equalized in concentration in the various body fluids. The diffusion coefficients of urea and of glucose at 15°C. are 0.94 and 0.52 respectively, indicating thus that urea is almost twice as diffusible as glucose. The studies of Bollman, Mann and Magath have indicated that the principal source of urea is the liver (4). This fact, even if correct, is no indication that urea may not likewise be formed, at least to some extent, in an inflamed area. Such studies had not previously been performed. That urea may be formed outside the liver is indicated, for instance, by recent studies showing that the mammary gland is capable of forming urea (5). The work of Krebs and Henseleit has suggested that liver alone is capable of forming urea (6). The mechanism involves the presence of ornithine in this organ. Ammonia and carbon dioxide combine with ornithine to form citrulline which ultimately is converted with the aid of additional ammonia into arginine. The latter in the presence of arginase rapidly forms urea and ornithine. On the other hand, Leuthardt has recently shown that urea can also be formed from glutamine and NH_3. Such synthesis is not catalyzed by ornithine (7). Bach has essentially confirmed and extended the studies of Leuthardt (8). Thus there are two distinct views on the possible formation of urea. It is therefore quite possible that this substance can be formed at the site of acute tissue injury without necessarily resorting directly to the mediation of the contained amino acids of the liver. The data published in the preceding study (1) in addition to the present observations are not incompatible with such a possibility. This

would substantiate further the concept of local gluconeogenesis from the products of enhanced proteolysis at the site of an acute inflammation in a diabetic animal. Further information, however, is still desirable before this important inference can be definitely established as a proven fact.

It is known that glycogen granules have been found in the leukocytes of diabetic patients (9). Is the high glucose concentration found in exudates of diabetic dogs the result of local metabolic disturbances liberating thus sugar from cells injured *in situ* or is this substance merely released by leukocytes which have infiltrated in the inflamed area from the blood stream? There is but little doubt that leukocytes are not essential elements in the process of gluconeogenesis at the site of inflammation in diabetic animals. This can be demonstrated by cytological studies of the exudate at the beginning of the inflammatory reaction. In the first few hours the exudate is found wholly devoid of leukocytes. It contains merely a few scattered desquamated mesothelial cells, doubtless derived in large part from the injured pleura. Yet, a comparison of exudate and blood sugar reveals a conspicuous concentration gradient. The acellular picture, as far as leukocytes are concerned, in the first few hours of inflammation indicates that gluconeogenesis is probably referable to cells injured *in situ* by the presence of the irritant. This would indicate that leukocytes *per se* are not necessary to explain the mechanism of gluconeogenesis involved. It is conceivable, however, that in an inflammatory exudate of about twenty-four hours' duration which at that time contains large quantities of granulocytes, the absolute level of glucose may perhaps be somewhat elevated and thus reinforced by the additional presence of leukocytes.

II. *Observations on non-diabetic dogs.* To control the foregoing observations, similar experiments were repeated on non-diabetic dogs with superimposed pleural inflammation. Samples of exudates were periodically withdrawn by thoracentesis. Blood specimens were obtained by cardiac puncture as described elsewhere (1). The data appear in table 2. An analysis of the measurements reveals in the first few hours of inflammation a concentration of glucose in the exudate that is considerably higher than in the blood stream. This initial heightening in the level of exudate sugar is reflected to some extent in an increase in blood sugar in at least two of the animals. Contrary, however, to the findings in the diabetic animals the enhanced exudate sugar is not sustained, but soon drops to a level which may be even lower than that of the blood sugar (table 2; chart 1). Reinjection of the irritant induces again a transient rise in exudate sugar. These findings differ from the results observed in diabetic animals in so far as in the latter the exudate sugar is maintained at a higher level than the blood sugar throughout the duration of the experiment (cf. chart 1). Furthermore, the apparent continuous production of sugar at the site of inflammation in a diabetic animal is reflected in the circulation where a gradual heightening in blood sugar thereby develops.

Is the transient increase in exudate sugar in non-diabetic dogs referable primarily to an accumulation of this substance from the circulating blood owing to local increased capillary permeability? That this may be in part the state of

affairs is not contended in view of the well known seepage of material from the blood stream into an inflamed area (10). The results in the case of dog 9 (table 2), for instance, substantiate such an interpretation. Furthermore, the inability of glucose to be fixed or concentrated in an area of acute inflammation would reasonably explain the transitory augmentation in exudate sugar of non-diabetic animals as an initial penetration from the circulating blood (3). It is difficult, however, to accept this explanation as the only mechanism involved in view of certain discrepancies. Examination of the data obtained in dogs 7 and 8 reveals

TABLE 2

The sugar and urea concentration in exudate and blood of non-diabetic dogs

DOG NO.	APPROXIMATE DURATION OF INFLAMMATION	EXUDATE SUGAR	BLOOD SUGAR	EXUDATE UREA	BLOOD UREA
	hrs.:mins.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
7	0		84.7		29.0
	1:30	189.6	120.3	43.0	37.0
	3:30	292.0	180.6	48.0	43.0
	25:25	68.6	114.9	30.0	28.0
8	0		100.0		
	2:00	248.5	151.0		
	6:30	70.6	85.5	67.0	58.5
	27:13	59.7	82.7	37.5	37.0
Reinjected irritant					
Interval after reinjection of irritant	1:47	288.7	117.6	23.0	
	5:27	60.3	110.2		
9	0		82.3		54.5
	2:00	125.9	76.9		
	3:40	167.4	95.7	75.0	64.5
	6:04	78.9	59.5	56.0	47.0
	24:44	65.7	55.8	30.5	24.5
	46:36	128.6	81.2	37.0	29.5
Reinjected irritant					
Interval after reinjection of irritant	1:49	155.7	86.4	36.0	31.5
	5:52	40.4	76.7	55.5	52.0
10	0		92.2		13.5
	2:05	134.2	102.6	23.0	22.0
	4:40	64.5	91.4	17.0	15.0
	26:28	97.6	114.3	31.0	23.0
	47:10	97.3	104.5	18.0	14.5
(Partially depancreatized) 11	0		99.8		16.5
	1:55	178.6	107.3	30.0	27.5
	5:15	119.4	96.3	23.5	23.5
	22:45	91.1	101.3		

that the initial rise in exudate level is reflected likewise in the blood sugar. If the transient augmentation in exudate sugar in non-diabetic animals is solely referable to an accumulation from the circulation, it is difficult to explain a concomitant tendency for the blood sugar to rise. One would expect a drop, if anything, unless one postulates the entrance of an immediate over compensatory mechanism on the part of the liver to increase the blood sugar level. There is no available evidence for the latter supposition, but nevertheless it is necessary to bear it in mind pending the repetition of these experiments on hepatectomized animals. At any rate on the basis of earlier observations on the penetration of material into an inflamed area (10), the accumulation of sugar from the circula-

tion would certainly be maintained for a considerable interval; and therefore if the liver played a significant compensatory glycogenolytic rôle this might be perhaps expected to be sustained. Yet the rise in blood sugar is only transient. In view of these facts it is therefore quite likely that the initial increase in exudate sugar in non-diabetic animals is also referable, at least in part, to local gluconeogenesis which in turn may be reflected in the blood stream by diffusion of glucose from the site of inflammation.

It is important to explain the transient nature of the rise in exudate and blood sugar in non-diabetic animals in contrast to the sustained effect in depancreatized dogs. In an earlier study it had been shown that the rate of glycolysis in an inflamed area is conspicuous, and in fact that it is considerably more marked than in the circulating blood (11). The gradual production of lactic acid thus adequately explains the mechanism of the developing local acidosis in an area of acute inflammation. It is therefore conceivable that in the inflamed area of a non-diabetic animal, gluconeogenesis at first transcends the initially mild glycolytic process. At a later stage, however, the latter reaction dominates the picture and in turn overshadows the effect of glucose formation. According to this view, gluconeogenesis is thus ultimately masked by glycolysis in the area of acute inflammation of a non-diabetic animal. The following data on two dogs seem to support such an interpretation:

DOG. NO.	DURATION OF INFLAMMATION	EXUDATE SUGAR	EXUDATE LACTIC ACID
	hrs.:mins.	*mgm./100 cc.*	*mgm./100 cc.*
7	3:30	292.0	17.8
	25:25	68.6	35.2
9	1:49 (after reinjection of irritant)	155.7	25.7
	5:52 (after reinjection of irritant)	40.4	95.5

It is clear that with the transient high exudate sugar in the initial phase there is a corresponding low concentration of lactic acid. The reverse order is found in the later stages of the inflammatory reaction. In brief, these facts probably explain satisfactorily, at least, in part, the initially elevated glucose level in the exudate of non-diabetic animals. It is, however, to be remembered that the final picture is doubtless somewhat altered, at least in some animals, by the diffusion from the blood stream of some glucose into the area of acute injury. In the diabetic dogs, on the other hand, there is an exaggerated degree of gluconeogenesis from non-carbohydrate precursors. Even though the process of glycolysis is distinctly more pronounced than in the exudate of non-diabetic animals, the overproduction of sugar dominates the picture inducing thus a sustained effect. This interpretation is fully substantiated by previous observations (1).

A comparison in the lactic acid and sugar levels in exudates of diabetic and non-diabetic animals indicated a rise of only 52 per cent in lactic acid and one of 473.6 per cent in the sugar of diabetic exudates (1). The facts summarized above support further the interpretation of various authors that carbohydrate metabolism in diabetes is merely a quantitative exaggeration of a normal process (12). The sum total of all observations on the trend of the exudate and blood sugar in both diabetic and non-diabetic animals is conveniently represented graphically in chart 1.

TABLE 3

The protein content of exudate and of serum in diabetic and non-diabetic dogs

DOG. NO.	APPROXIMATE DURATION OF INFLAMMATION	TOTAL PROTEIN OF EXUDATE	TOTAL PROTEIN OF SERUM	DOG NO.	APPROXIMATE DURATION OF INFLAMMATION	TOTAL PROTEIN OF EXUDATE	TOTAL PROTEIN OF SERUM
Experimental group (diabetic animals)				Control group (non-diabetic animals)			
	hrs.: mins.	*gm. per 100 cc.*	*gm. per 100 cc.*		*hrs.:mins.*	*gm. per 100 cc.*	*gm. per 100 cc.*
1	6:13	5.3	6.2	7	3:30	9.1	5.3
	25:00	4.6	5.6		25:25	4.9	4.5
5	4:30	4.6	5.5	9	3:40	10.8	6.6
	23:20	4.8	4.7		6:04	7.7	6.6
6	2:00	3.3	5.5		24:44	4.9	7.5
	46:55	3.4	4.9		46:36	4.7	6.0
				10	2:05	8.5	4.3
					4:40	5.3	4.6
					26:28	4.2	4.4
				11	5:15	9.5	6.5
				8*	2:00	4.0	6.9
					6:30	5.5	7.0
					27:13	4.8	5.7

* This animal's protein metabolism seemed to be atypical from the very beginning. For instance, prior to the introduction of the irritant, its blood urea was excessively elevated, namely, 87 mgm. per 100 cc. and its NPN was 58.1 mgm. These high figures were maintained immediately after the introduction of the irritant. Two hours after the onset of the inflammatory reaction the blood urea was 102.5 mgm. per 100 cc. and the exudate urea was 110 mgm. The non-protein nitrogen was 71.9 mgm. in the blood and 65.6 mgm. in the exudate. These unusually elevated base levels in the constituents of protein catabolism may well account for the inconsistent findings in the total protein content encountered in this particular animal.

The foregoing observations suggest that in an area of acute inflammation in a non-diabetic animal there is a certain amount of gluconeogenesis. If the glucose formed locally originates from protein breakdown processes as in the case of diabetic dogs, proteolysis and therefore a somewhat elevated urea level in the exudate might be expected to occur. The data appear in table 2. It is clear that the urea concentration of exudates is a trifle higher than that found in the blood. The difference in levels though definitely small is nevertheless consistent.

III. *Proteolysis in inflamed areas of diabetic and non-diabetic animals.* It is known that the inflammatory reaction is accompanied by proteolysis (13, 1).

Furthermore, previous studies had demonstrated that the local protein breakdown processes in an area of acute injury of a diabetic animal are distinctly enhanced, thus offering a reasonable explanation for the excessive formation of

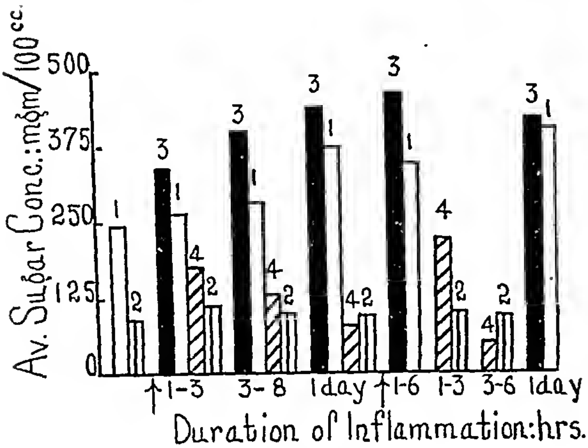

Chart 1

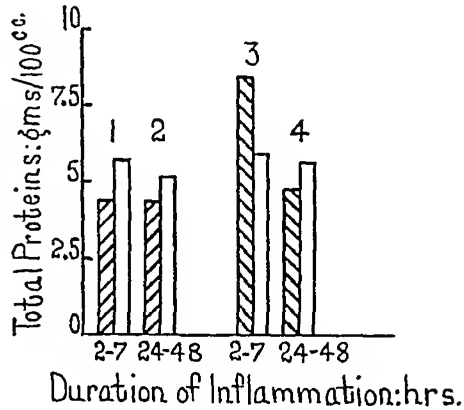

Chart 2

Chart 1. Composite data showing the trend of the exudate and blood sugar levels in diabetic and non-diabetic dogs.

Column 1 represents average blood sugar in diabetic dogs.

Column 2 represents average blood sugar in non-diabetic dogs.

Column 3 represents average exudate sugar in diabetic dogs.

Column 4 represents average exudate sugar in non-diabetic dogs.

The first arrow on the left represents the time of injection of the irritant. The arrow to the right represents the time of reinjection of the irritant. The time indicated after the second arrow on the right refers to the interval which has elapsed following the reinjection of the irritant.

Chart 2. Average protein content of exudate and of serum in diabetic and non-diabetic dogs. Note the intense degree of proteolysis in the initial stage of inflammation in diabetic animals. As a result, the protein content of exudates is lower than that of blood serum. On the contrary, in the non-diabetic animals the process is considerably milder. The effect of increased capillary permeability at the site of inflammation in the earliest stage of the reaction transcends the relatively milder proteolytic process. The consequence is an outpouring of proteins from the circulation into the area of injury, and therefore the protein content of the exudate temporarily rises to an even higher level than that found in the blood serum. Subsequently the extent of local proteolysis overcomes this initial effect and the total protein content of exudates drops to a level below that of the serum proteins.

The protein content of the exudate of diabetic animals is represented in the columns with diagonal lines drawn downward from right to left.

The protein content of the blood serum of diabetic and non-diabetic animals is represented in the blank columns.

The protein content of the exudate of non-diabetic animals is represented in columns with diagonal lines drawn downward from left to right.

Columns labelled 1 and 2 refer to the material studied in diabetic animals.

Columns labelled 3 and 4 refer to the material studied in non-diabetic animals.

sugar from non-carbohydrate precursors (1). A study was undertaken to compare the protein content of exudate and of blood in diabetic and non-diabetic dogs at varying stages of the inflammatory reaction. The observations are conveniently assembled in table 3 and in chart 2.

In the experimental group of depancreatized dogs the total protein level of exudates is distinctly lower than that of blood serum. It is of significance to note that this difference occurs in the earliest stages of the reaction. For instance, an inflammation of two hours' duration reveals a lower protein level in the exudate than the corresponding level in the serum. Such evidence indicates a high rate of proteolysis in the exudate of diabetic dogs. The effect of the increase in capillary permeability which would favor the seepage of plasma proteins from the circulation into the area of injury (14), and thus elevate the level of proteins in the exudate is evidently nullified by the rapid and enhanced rate of local protein breakdown. The result is a lowering in the concentation of total proteins in exudates of diabetic dogs from almost the inception of inflammation.

These findings stand in sharp contrast to the observations recorded on the exudates of non-diabetic animals (table 3 and chart 2). It is clear that in the latter at the beginning of the inflammation the total proteins are considerably elevated. The level is distinctly higher than that in the blood serum. This difference is doubtless referable to an early enhanced capillary permeability favoring the passage of proteins from the blood into the area of acute injury (14). Subsequently, the developing proteolysis in the inflamed area transcends this effect and the protein level of exudates becomes definitely lower than that of the serum. For instance, in dog 9, table 3, the total protein content of the exudate is 10.8 grams per 100 cc. when the inflammation is slightly over three and a half hours' duration in comparison with a concentration of 6.6 in the serum. After an interval of about twenty-four hours proteolysis evidently becomes a conspicuous part of the inflammatory reaction. At that time the total protein content of the exudate measures 4.9 grams as compared with 7.5 grams in the serum. These facts indicate that there is a more pronounced degree of proteolysis in the exudate of a diabetic than in that of a non-diabetic dog. This finding adds further support to the view that the greater local gluconeogenesis in the inflamed area of diabetic animals is associated with enhanced proteolysis.*

Discussion. Soskin regards carbohydrate metabolism as a dynamic balance between blood sugar formation by the liver and its utilization in the tissues (15). According to this investigator the height of the blood sugar level itself regulates the degree of inhibition on the part of the hepatic mechanism. Furthermore, Soskin and Mirsky (16) showed that intravenous administration of diphtheria toxin to a dog alters the normal dextrose tolerance curve. The abnormality was referred to injury to the liver. The blood sugar tends to be elevated apparently due to an augmented hepatic glycogenolysis. Can the present observations on non-diabetic animals be referred primarily to such hepatic involvement? In the initial stage following its injection into the pleural cavity some turpentine is probably absorbed into the circulation. This material may, by reaching the liver, injure this organ and thus induce changes in the blood sugar. The present

* As shown in the earlier study (1) the administration of insulin to diabetic animals with superimposed inflammation represses not only local gluconeogenesis in the injured area but likewise the enhanced proteolysis, indicating thus that the formation of glucose is apparently referable to the proteolytie process.

evidence does not fully preclude such a possibility. However, it is to be noted that preliminary studies on the dextrose tolerance curve in a normal dog and in a dog with pleural inflammation induced by turpentine have failed to reveal any appreciable difference. Hepatectomized preparations to be studied in the future may possibly supply additional information on this point. The observations of the data obtained as shown in tables 1, 2 and 3 definitely suggest the plausibility of a different interpretation. In the first place, essentially throughout the duration of inflammation the exudate sugar in diabetic animals is at a consistently higher level than the blood sugar (table 1, chart 1). This occurs in spite of the fact that this substance is extremely diffusible and as shown by Miller (3) cannot be fixed or concentrated at the site of inflammation. In the second place, whereas the initially elevated blood sugar in non-diabetic dogs may favor the interpretation of an increased formation of glucose by the liver (cf. dog 7, table 2), it is difficult to explain on this basis some of the other results which show a relatively elevated exudate sugar and yet an essentially unaltered level of blood sugar (cf. dogs 9 and 10, table 2). Therefore, in view of these facts, it seems more reasonable to interpret the mechanism involved as primarily one of gluconeogenesis at the site of inflammation. When the amount of glucose formed is conspicuously high, some of the material diffuses readily into the circulation. A concentration gradient is thus established characterized by a higher exudate than blood sugar level. When the process of gluconeogenesis at the site of inflammation is not as marked, one may encounter merely an elevated exudate sugar with, however, no appreciable rise in blood sugar (dogs 9 and 10, table 2). In brief, the available data strongly suggest that basically the same mechanism occurs at the site of injury in the non-diabetic as in the diabetic animals. In the latter the process of gluconeogenesis is merely exaggerated. In the non-diabetic animals glycolysis in the inflamed area (10) eventually supervenes and thus tends to obliterate the effect of the initial gluconeogenesis.[3] In the diabetic animals glycolysis is also enhanced, but not to the same extent as glucose production (1). The result in the depancreatized animal is a marked increase in local gluconeogenesis at the site of inflammation; the glucose gradually diffuses into the circulation. Thus the inflamed area seems to behave as an accessory focus of gluconeogenesis from non-carbohydrate precursors simulating in this respect the function of the liver. This does not mean that the hepatic mechanism may not have some influence in regulating, especially in non-diabetic animals, the transitory, initially exaggerated hyperglycemia which may occur as a result of a superimposed inflammation; but any such effect is presumably secondary to the primary mechanism at the site of acute injury. Finally, accumulation of glucose from the circulation into the acutely inflamed area due to increased capillary permeability doubtless affects somewhat the ultimate level of sugar in the exudate; but the diffusibility of this substance as well as the increase in blood sugar

[3] It is quite conceivable that the various degrees of hyperglycemia and glycosuria or the lowered carbohydrate tolerance accompanying numerous unrelated infectious conditions may perhaps have as a common basis local gluconeogenesis by injured cells with subsequent diffusion of glucose into the circulation (17, 18, 19, 20).

observed in some instances concomitantly with the elevated exudate sugar of non-diabetic animals would preclude the rise in exudate sugar as being due primarily to local accumulation from the blood stream. Diffusibility, increased capillary permeability and therefore penetration from the blood stream into the inflamed area, and local glycolysis are all factors which are to be considered but none of them adequately explains the basic mechanism apparently involved, namely local gluconeogenesis by injured cells.[4]

CONCLUSIONS

An acute inflammation in a depancreatized dog is accompanied by a marked degree of local gluconeogenesis. The surplus glucose formed in the inflamed area from products of local protein breakdown diffuses into the circulation, enhancing thus the existing state of hyperglycemia. The concentration of exudate sugar is at a consistently higher level than the blood sugar from the very beginning of the inflammatory reaction. When the inflammation has progressed for about one day, the concentration of blood sugar tends to approach that of the exudate sugar. The establishment of a concentration gradient between the level of exudate and blood sugar strongly supports the view of a gluconeogenetic process at the site of inflammation.

A similar gradient, though not as marked, is found to exist between the urea concentration of exudate and that of blood. The difference in the magnitude of the glucose and urea concentration gradient seems to be primarily referable to the respective diffusion coefficient of these two substances. The extent of proteolysis in the exudate of a diabetic dog is definitely more marked than in that of a non-diabetic animal (table 3). These facts add further support to previous observations that gluconeogenesis in the inflamed area of a diabetic animal is associated with enhanced local protein catabolism (1). The process of local glucose formation is not primarily referable to the presence of leukocytes but rather to cells in general injured at the site of inflammation.

In a non-diabetic animal the concentration of sugar in exudate is at first higher than that in blood. This effect, however, is transient. This, in turn, is contrary to the findings in diabetic dogs. After the inflammation has progressed from several hours to about a day, the sugar level in exudate of non-diabetic dogs drops to a level usually below that of the blood sugar. This lowering in exudate

[4] It may be of interest to determine whether the amount of sugar capable of being formed in an area of acute pleural inflammation in a diabetic dog can reasonably account for the rise in the hyperglycemia of such animals. An estimate of gluconeogenesis made on the basis of an average dog weighing about 8 kgm. and having an inflamed pleural cavity containing a total protein content in the exudate of about 4 per cent yields approximately 2320 mgm. of glucose per 100 cc., i.e., if all the proteins had been deaminized in the conversion to glucose. Such a quantity of sugar diffusing into the circulation from the site of inflammation would augment the blood sugar by over 330 mgm. per 100 cc. Inasmuch as previous studies indicate an increment of about 216 mgm. of dextrose per 100 cc. of blood in diabetic animals with superimposed pleural inflammation, it is clear that there is an ample marginal reservoir of sugar liberated in the inflamed pleural cavity of a diabetic dog to account for the enhancement in hyperglycemia (1).

sugar concentration is referable to an increase in the local glycolytic reaction which thus overshadows the initial effect of glucose formation at the site of an acute inflammation. If, at the beginning of inflammation in a non-diabetic animal, the degree of local gluconeogenesis is marked, the effect may be reflected in the circulation inducing thus a transient hyperglycemia. The available evidence suggests that the temporary elevation in exudate sugar in the inflamed area of a non-diabetic animal is primarily referable to local gluconeogenesis. The difference in reaction from that in diabetic dogs is quantitative in nature. In the latter local gluconeogenesis is sustained and exaggerated. The effect of abundant glucose production in depancreatized animals cannot be readily obliterated by the slightly elevated local glycolysis. The consequence is constant gluconeogenesis; the glucose in turn diffuses into the circulating blood, thus enhancing the diabetic condition. In brief, injured cells, as manifested by inflammation in both diabetic and non-diabetic animals, are characterized by an increase in their protein catabolic processes and by potentially becoming foci of gluconeogenesis.

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THE OXYGEN CONSUMPTION OF THE SKIN DURING THE HAIR CYCLE IN THE WHITE RAT

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Hair growth in the white rat is cyclic, the follicles being active for about sixteen days and inactive for a similar period (1). This cyclic activity may be demonstrated histologically or it may be observed by removing the hair from the back with a depilatory agent at the end of the growing period (age 20 days) and then watching hair eruption externally about the 35th day of life.

Experiments have been conducted for sometime in learning what factors influence the growing and resting stages of the hair follicle. It has been found that the follicles will remain inactive for long periods if rats are fed only enough feed to prevent a loss in body weight (2). If small amounts of chloral hydrate (10–20 mgm.) are administered subcutaneously daily to well fed animals, the hair buds also remain inactive.

Hair growth can be induced on the backs of underfed rats either by the daily administration of thyroxin (0.2 mgm. subcutaneously for 3 days) (3) or by the daily irritation of the skin on the back with some irritant (xylene, benzoic acid, capsicum, cantharides) (3).

When one analyzes these various results, it appears that hair growth resulted either from an increased passage of food or exciting substances through the capillary wall to the hair follicle, or from increased oxidative processes. Other experiments (4) show that the accelerated hair growth following adrenalectomy is not due to the increased passage of fluid.

It seemed appropriate at this time to determine the oxygen consumption of the skin at various intervals during the hair cycle and to see if there was any evidence that oxidative processes influenced the activity and inactivity of the hair follicle.

METHOD. Hair was removed from the back of the rats with sodium sulphide several days prior to the time of determining the oxygen consumption of the skin so that the depilation would not influence the activity of the skin.

When the determinations were to be made, the rat was killed by a blow on the head and uniform slices of skin (approximately 0.5 mm. in thickness and 1 cm. long) were taken from the dorsum. The oxygen consumption of these slices, usually four, was measured volumetrically in air by means of a Fenn microrespirometer.[1] The slices were shaken in Ringer's-phosphate solution (pH—7.2) at a temperature of 37.5°C. Both the tissue and differential flasks of each respirometer contained 1 cc. of medium, and five drops of N/5 $Ba(OH)_2$ were placed in the small compartment of the tissue flask to absorb the carbon dioxide. Four respirometer tubes were usually run at the same time. Their entire prepa-

[1] I am greatly indebted to Dr. J. A. Dye of the Department of Physiology of Cornell University for the use of microrespirometers and other apparatus.

ration required about twenty minutes, and an interval of twenty minutes was allowed for temperature equilibration before the first reading was taken. After the tests were run for two hours, the tissues were dried to a constant weight at 110°C. Results are expressed in cubic millimeters of oxygen consumption per milligram of dry tissue per hour. The amount of tissue taken usually weighed about 10 mgm. after desiccation.

Results. Determinations of the oxygen consumption of the skin of different rats were made daily during the inactive period (20th to 32nd days) of the hair follicle so that changes could be detected in the oxygen consumption of the skin at the conclusion of the growth of the follicle and prior to the beginning of activity in the hair bud.

Two microrespirometers yielded rather consistent and comparable results for the first hour throughout the experiments. Since they correlated so closely, the data for them are described in detail. Figure 1 shows the results in tube A for the first hour during the various days. The skin from three different rats aver-

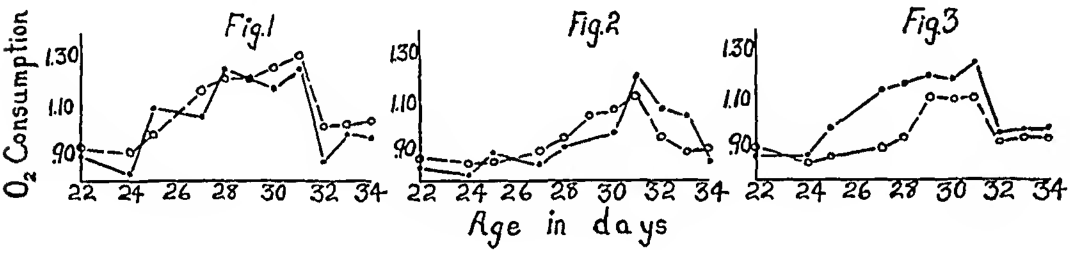

Fig. 1. Oxygen consumption during the first hour. Tube A ·———; tube B o- - -.
Fig. 2. Oxygen consumption during the second hour. Tube A ·———; tube I o- - -.
Fig. 3. Average of all determinations in four tubes. First hour ·———; second hour o- - -.

aged 0.92 cmm. of oxygen per milligram of dry tissue on the 22nd day of life. On the 24th day the skin from four other rats averaged 0.84 cmm. per mgm. This decrease might be due to lowered metabolism following weaning. Oxygen consumption then increased rapidly, reaching 1.26 cmm. per mgm. on the 28th day, or about a 30 per cent increase over that on the 22nd day. The greatest consumption usually occurred on the 31st day. A decrease began after the 31st day. Active growth starts in the hair bud about the 31st day and cells are added rapidly thereafter. The skin is even using more oxygen on the 32nd day than it is on the 30th day. The weight of the skin, however, is being increased by the addition of dead hair cells, and the oxygen consumption per milligram of skin accordingly decreases. Each day's results in figure 1 represents the average of the determinations for at least three rats.

In general the determinations in tube B (fig. 1) follow closely those made in tube A. Again each day's record is the average of the determinations for at least three rats, the same ones which were investigated by the use of tube A. For instance, compare the results in tube A on the 31st day (1.28 cmm. per mgm.) with the average in tube B (1.31 cmm.) for the same three rats.

A study of the determinations in tubes A and I during the second hour (fig. 2) shows a gradual increase of oxygen consumption on the successive days up to the 31st day. Both of these tubes closely correlate, and both show a decline after the 31st day.

Changes in the oxygen consumption of the skin of the individuals of two litters chosen at random may be followed. The results at the various ages represent the average of the determinations in four tubes.

In litter 10 (table 1) the greatest consumption during the first hour is at the age of thirty days. Note the very gradual increase at the various ages. Histological

TABLE 1

Oxygen consumption of the skin of individuals of litter 10

AGE	OXYGEN CONSUMED IN CUBIC MILLIMETERS PER MILLIGRAM OF TISSUE (DRY)		
	First hour	Second hour	Average
days			
24	0.87	0.68	0.78
25	1.00	0.78	0.89
28	1.07	0.93	1.00
30	1.09	0.95	1.02
32	0.90	1.00	0.95
34	0.90	0.85	0.87

TABLE 2

Oxygen consumption of the skin of individuals of litter 11

AGE	OXYGEN CONSUMED IN CUBIC MILLIMETERS PER MILLIGRAM OF TISSUE (DRY)		
	First hour	Second hour	Average
days			
22	0.89	0.93	0.91
24	0.98	1.00	0.99
26	0.94	1.05	1.00
29	1.53	1.35	1.44
31	1.18	1.21	1.19
33	1.02	0.88	0.95

sections of the skin taken on the thirtieth day show no growth whatsoever in the hair buds. Therefore, the oxidative processes increase prior to growth in the hair bud. In litter 11 (table 2) an enormous increase occurs on the 29th day of life. Sections of the skin taken on this day, likewise, show no mitotic activity in the hair bud.

The average of all determinations is shown in figure 3. Each day's record represents the average of at least twelve determinations made in four different tubes. During the first hour the consumption increases rapidly from the 24th day to the 29th day. During the second hour the increase is more gradual. The consumption for the second hour as may be expected is less than that for the first hour.

DISCUSSION. These experiments show that oxidative processes increase prior to activity in the hair follicle. Since the oxidative processes of the skin of underfed animals do not increase and the hair does not grow (unpublished data), it would seem that oxidative processes greatly influence growth, or an increase in the anabolic phase of metabolism is a forwarder of growth. It is entirely possible that the follicle completes growth and oxidative processes awaken it from inactivity.

Processes of maintenance take precedence over those of anabolic growth, and this explains why the hair does not grow upon underfeeding. When underfed animals are treated with either thyroxin or irritants, the oxidative processes or anabolic phase favors growth even with the demand for maintenance. The maintenance demand is not an all out demand or so intense that the animal seriously loses weight. In other words the call of maintenance is not so great but that some substance can be diverted to hair formation.

Thyroxin favors growth in the underfed and well fed animals. From these experiments this would seem to be due to an increase of oxidative processes which the thyroxin causes (5). Others (6) would be inclined to think that the cellular specialization or differentiation in the hair follicle is due to the tyrosine radicle which the thyroxin contains. Administration of 50 mgm. of diiodotyrosine subcutaneously daily for many days in underfed rats failed to induce hair growth. Likewise, 100 mgm. of 1-tyrosine daily had no effect. Feeding of 1-cystine (40 mgm. daily) also failed to induce growth.

SUMMARY

During the cyclic hair growth in the rat, it has been found that the skin at the conclusion of activity in the hair bulb (age 22 days) is consuming 0.92 cmm. of oxygen per milligram of dry tissue per hour. The oxygen consumption gradually increases and just prior to activity in the hair follicle on the 31st day of life, the skin is consuming 1.31 cmm. per hour. Oxidative processes do not increase in the skin of the underfed animals and the hair doesn't grow. Increase in oxidative processes or the anabolic phase of metabolism just prior to active growth in the hair bud is, therefore, believed to favor the growth of hair in the normal rat.

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THE EFFECT OF AN ENCIRCLING CONDUCTING BAND UPON THE ACTION CURRENTS OF STRIATED MUSCLE

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It has long been recognized that the conductivity and distribution of the material surrounding a muscle may have a profound effect upon the shape of the recorded action potential. That such effects may be fundamental causes for certain alterations of the electrocardiogram has been stressed, particularly by Katz and his collaborators. The effects of immersion in a continuous conducting medium or of a film of conducting material surrounding the muscle form an important part of the now classical study of Craib on the fundamental nature of the electrocardiogram.

These workers have considered the effect of the external medium upon the action current curves for cases in which the medium under consideration surrounds the muscle and has contact with the electrodes.

This report is concerned with the alterations of the action current curves caused by changes in the medium surrounding the muscle which do not directly affect the region of the electrode contact.

The muscles used in these experiments are the retractor capitis muscles of the common turtle. These are long cylindrical muscles inserted in the region of the head with origin at the caudal end of the carapace. The animals were curarized before dissection.

These muscles were supported isometrically in air by means of strings tied to each end. Action currents were recorded with non-polarizable electrodes near each end. The stimulus for the contraction was obtained by the discharge of a small condenser (0.25 microfarad) across the muscle at the end nearer that electrode negativity of which produced an upward deflection. The recordings were made with a cathode ray oscillograph and amplifier, the stimulus being released by a relay suitably arranged in the time sweep circuit of the oscillograph. The amplifier used is condenser coupled with an effective time constant of over 2 seconds.

Tracing A 1 shows a typical record of this kind. The action current wave is a simple biphasic wave. There is no isoelectric interval.

If, now, an encircling band of cotton, about 1.5 cm. wide, soaked in Ringer's solution, a "collar," is placed around the muscle, midway between the electrodes, a profound change is noted in the record. The initial wave is now splintered (tracing A 2). This may be explained simply as a short-circuiting of the advancing doublet while in the region of the conducting collar. Upon removal of the collar the wave returns to the original condition (tracing A 3). The wave of tracing A 1 and that of tracing A 2 are practically identical except for the sharp

dip, as if a portion of the wave were eliminated or suppressed. Similar effects have been observed and similarly explained in nerves by Marmont (1).

In order to demonstrate the correctness of the proposed explanation the following experiment was performed. A single broad collar extending along a segment 6 cm. long was placed about the muscle midway between the electrodes. The resulting action current is shown in tracing B 1. The broad collar was then removed and replaced by two collars 2 cm. wide, placed 2 cm. apart. The result is shown in tracing B 2. These collars were then connected with a salt bridge, i.e., a piece of saline soaked cotton connecting both collars without touching the muscle. The result is shown in tracing B 3. A metal connection

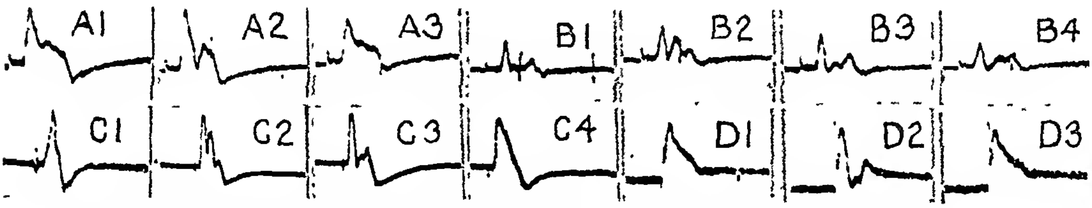

Fig. A 1. Action current of retractor capitis muscle of turtle.

A 2. Same with Ringer's solution collar 1.5 cm. wide midway between electrodes.

A 3. Collar removed.

The time scale of all tracings is 10 cm. per second. Short vertical time marks (0.1 sec.) may be seen on some of the records.

Fig. B 1. Action current with 6 cm. collar.

B 2. Action current with two 2 cm. Ringer's solution collars, with 2 cm. uncovered segment between them.

B 3. Same as B 1 but with saline soaked cotton bridging the gap between the collars, but not touching the muscle.

B 4. Same as B 3 but with metal connection instead of saline bridge.

Fig. C 1. Action current of muscle alone.

C 2. Action current with two Ringer's solution collars.

C 3. Same as C 2 but with collars connected by metal strip not touching muscle.

C 4. Collars removed.

Fig. D 1. Action current of folded muscle with contact of two portions avoided.

D 2. Same as D 1, but with contact.

D 3. Contact again prevented.

of both collars in place of the salt bridge resulted in the wave shown in tracing B 4. In each case after removal of the collar the original wave was again obtained. The effect is thus purely electrical. The collars of Ringer's solution produce no change in the excitation phenomena, but a pronounced change in the electrical manifestations is observed. Another example is shown in tracings C 1–4. Similar collars of tin foil instead of saline produce the same effect. Collars of distilled water or dry collars have no effect.

The question arises as to whether these effects can throw light on the observed predominance of the surface of the heart in determining the form of the electrocardiogram (2, 3). The collar placed about the muscle can obviously have no effect upon any part of the muscle other than its surface. The observed phenomena are another expression of the basic fact that the action currents re-

corded from complete muscles are due to the effects at the muscle surface. This is true no matter what the localization of the actual action potential gradient, whether caused by dipole distributions along the surface of the fibres or dipole distributions across the fibres. The short-circuiting action of the collar may be expected to be the same whichever localization actually prevails in the individual fibre.

Collars such as were used in these experiments may in the future be used as detectors of the passage of excitation for the purpose of velocity measurements. Such a method may, under certain conditions, be much simpler to apply than the usual procedures involving multiple recording.

A single wide collar suppresses a large portion of the initial wave. Thus such a collar results in an action current wave which has two erect components and bears a tantalizing resemblance to the electrocardiogram with its erect R and T wave (tracing B 1). Effects similar to that produced by a wide collar or by two connected collars may be produced by bending the muscle back on itself around an insulating support so that a segment is short circuited by contact. Tracings D 1–3 show the recorded waves for such a case. In tracing D 1 the contact is broken by holding the two portions apart. In tracing D 2 contact is established. In D 3 it is again broken. The R-T appearance is obvious. These results suggest the possibility that the well known twisted nature of parts of cardiac muscle may have some similar influence on the electrocardiogram. In the present unsatisfactory condition of T wave interpretation this possibility may be considered, but we do not believe this to be a complete explanation for various reasons, particularly because of the length of the R-T interval.

SUMMARY

Typical changes are produced in the action potential curves of striated muscle if the muscle be surrounded by a ring or collar of conducting material which does not impinge upon the electrodes. The effect is explained and is related to the predominance of the surface of the heart in the production of the electrocardiogram. The effect may be applied in the measurement of conduction velocity. A modification of the experiment permits the production of action potential curves from striated muscles which closely resemble the R-T of the electrocardiogram.

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REMOVAL OF RED CELLS FROM THE ACTIVE CIRCULATION BY SODIUM PENTOBARBITAL[1]

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It is commonly observed that the spleen under the influence of sodium pentobarbital (nembutal) becomes engorged and turgid. This is generally considered to be due to the trapping of red cells in the sinusoids. The use of red cells tagged with the[2] radioactive isotope of iron offers a direct means of determining what fraction of the circulating red cells of the body are contained in this organ under these conditions.

Studies of the various forms of shock must inevitably take into consideration the changes which occur in the circulating constituents of the blood as well as the other body fluids. Attention has centered chiefly on variations in plasma volume. Some of the plasma may at times be out of the active circulation (9) and methods employing dilution of dyes may under these conditions yield especially erroneous results. The red cells are *normally* nearly all in active circulation (8) (12) and the measurement of their circulating mass may be carried out by one of several procedures (1) (8). However many studies are made under anesthesia and it is important that the effect of such complicating factors be understood.

Two methods of approach were used in estimating the amount of red cells which might be taken up by the spleen under nembutal, one of these being specific and the other not so. In the first the circulating cells were tagged by injection of donor red cells containing radioactive iron in their constituent hemoglobin. Nembutal anesthesia was induced and the spleen allowed to become engorged. Splenectomy was performed and the organ was subject to wet ashing (7), the iron separated, electroplated, and its radioactivity determined. The total activity in the spleen divided by the concentration of radioactivity in the red cells gave directly the mass of cells in that organ.

The other method consisted in first[3] nembutalising the animal and allowing time for engorgement of the spleen to occur. The actively circulating mass of red cells was then determined by the donor-isotope-cell method (8) (9). The animal was then given a single large dose of epinephrine[4] ($\frac{1}{2}$ ml. of 1:1,000) by vein. After three minutes another sample of blood was drawn and the activity concentration of the circulating red cells determined. Unless circulation in the

[1] We are indebted to the Eli Lilly Company for aid in conducting this work.

[2] We wish to express appreciation to the members of the Radiation Laboratory at Berkeley, California, and particularly to Dr. E. O. Lawrence and M. D. Kamen for the radioactive iron used in these experiments.

[3] Veterinary nembutal, Abbott Laboratories.

[4] Suprarenin, Winthrop Chemical Company.

spleen is as rapid as that of the general circulation, cells for the most part in that organ would not be expected to be tagged under these conditions, and following adrenalin there should have been a further dilution of the circulating red cell radioactivity. This reaction is not specific for the spleen as we shall see below since it is subject to modification by any other part of the systemic circulation which might be affected in a similar manner by nembutal.

Blood from donor dogs was drawn variously into heparin, saturated sodium citrate (1.25 ml. per 100 ml. of blood used), or into isotonic sodium citrate since concomitant studies were being conducted on the survival time of transfused red cells using various anticoagulants. The blood samples of the recipients when taken for sampling were mixed with 5 ml. of isotonic oxalate, 30 ml. of blood usually being taken to allow triplicate hematocrit readings and activity measurements. Cells were centrifuged for 35 minutes at about 2700 r.p.m.

Methods for ashing of blood samples and spleens, separation of iron, and electroplating for determination of activity have been described (5) (7) (9).

EXPERIMENTAL OBSERVATIONS. Two dogs were first studied in order to determine the number of red cells contained in the spleen under ether anesthesia. The circulating red cell mass was determined by the isotope-donor-cell method (8) (9). Under ether the splenic blood vessels were then doubly ligated and the organ removed by cutting between the ligatures. After wet ashing the iron of the spleen was separated, electroplated, and the amount of radioactivity measured (5) (9). The total radioactivity of the spleen divided by the concentration of the isotope in the red cells of the circulation then corresponded to the volume of cells in this organ. In the case of dog 41-888, weighing 14 kgm., this amounted to 20 ml. of cells or 3.8 per cent of the total body cells. In the instance of dog 41–807, weighing 13.4 kgm. there were 19 ml. of cells in the spleen corresponding to 2.9 per cent of the total in the body.

Dog 1-G, 10.5 kgm. in weight, was studied to see directly how many red cells might be sequestered by the spleen under the influence of nembutal. A determination of the circulating red cell mass was made by the isotope-donor-cell method. Nembutal was then given by vein in anesthetic dose (27 mgm. per kgm.) and after allowing about seven minutes for the spleen to become engorged, a medial incision was made below the costal margin and the spleen excised as before. The organ was then wet ashed and the iron separated and measurement of the radioactivity done. The total radioactivity in the spleen divided by the concentration of red cell isotope again was a measure of the volume of cells taken up by this organ. In this dog the circulating cell mass was found to be 240 ml. of which 45 ml. or 18 per cent were taken up by the spleen.

Dog 36-196 was treated in exactly the same manner and the circulating cell mass was found to be 354 ml. of which the spleen under the influence of nembutal took up 101 ml. or 29 per cent of the total, table 1. Similarly the cell mass of dog 36-14 was found to be 320 ml. of which 58 ml. or 18 per cent were contained in the spleen.

Another approach was employed in the next few experiments. The dogs were first given the nembutal and after allowing time for the spleen to become en-

gorged with red cells, a determination of the *circulating* red cell mass was done. This latter measurement would presumably exclude the cells sequestered in the spleen provided the circulation rate in this organ were slow compared with the

TABLE 1

Removal of red blood cells from effective circulation by sodium pentobarbital

Direct determination of cells in spleen by splenectomy

EXP.	DOG		RED BLOOD CELL HEMATOCRITS: Initial	RED BLOOD CELL HEMATOCRITS: After inj. donor blood	RED CELL MASS: Total circl.	RED CELL MASS: Per cent in spleen
			per cent	*per cent*	*ml.*	*per cent*
1	1-G	Determination of cell mass tagging all circulating red cells; nembutalised; spleen ligated and excised, ashed and radioactivity measured	24.4	21.1	240	18
2	39-196		43.8	44.8	354	29
3	36-14		29.0	28.4	320	18
4	2-G		25.7	25.3	268	5

Indirect determination by cell mass dilution following adrenalin

EXP.	DOG		RED BLOOD CELL HEMATOCRITS: Initial	RED BLOOD CELL HEMATOCRITS: After inj. donor blood	RED BLOOD CELL HEMATOCRITS: After adrenalin	CELL MASS: Initial	CELL MASS: After adrenalin	PER CENT SEQUESTERED
			per cent	*per cent*	*per cent*	*ml.*	*ml.*	*per cent*
5	40-15	Nembutalised, sequestering red cells; effective circulating cell mass determined by donor-tagged-cell method; injected epinephrine increasing circulating cell mass, the increment being measured by further dilution of circulating cell activity	50.6	48.9	60.6	645	905	29
6	2-G		25.7	25.3	29.3	268	274	2
7	36-57		49.8	44.2	55.7	560	865	35
8	36-57		31.6	28.1	39.2	326	368	17
9	36-57		33.0	30.8	41.5	493	585	18
10	38-179		44.2	41.9	58.6	645	1045	37
11	38-179		35.0	33.7	41.3	402	610	34
		Splenectomised controls: Determination of cell mass increase after adrenalin						
12	39-196	Splenectomised dogs; nembutalised sequestering red cells; effective circulating cell mass determined by donor cell method; injected adrenalin, increasing circulating cell mass, the increment being measured by increased dilution of radioactivity of circulating red cells	42.5	42.4	43.8	304	355	14
13	1-G		25.5	25.8	28.0	204	242	16
14	2-G		23.0	22.8	24.2	240	271	11

rest of the vascular system. Adrenalin was then administered by vein in a single dose of ½ ml. (1:1,000) to cause contraction of the spleen. The resultant outpour of untagged red cells into the circulation resulted in a further dilution of the tagged cells already circulating. Another determination of the concentration of the red cell isotope resulted in a new value for cell mass (total

radioactivity of the injected donor cells divided by the concentration of the isotope in the circulating cells after allowing mixing in the blood stream-cell mass). The difference between the cell mass found before and after the epinephrine was taken to represent the mass of red cells held by the spleen and other tissues under the influence of the nembutal. In table 1 it can be seen that this amounted to 29 per cent of the red cells in dog 40-15; 35 per cent, 17 per cent, and 18 per cent on different occasions in dog 30–57; and 37 per cent and 34 per cent on two occasions in dog 38-179.

In one instance it was attempted to combine these two procedures. Dog 2-G was nembutalised, the spleen brought outside the abdomen, and the cell mass determined as before. It measured 268 ml. Adrenalin was injected and the spleen was seen to shrink down somewhat. No plethysmographic or other volume measurements were made however. Sampling of the blood showed that the cell mass had been increased only about 6 ml. which is well within the experimental error. After allowing a period of fifteen minutes for the epinephrine effect to wear off completely it was hoped that the organ would attain its former size again. Since there was no noticeable increase an additional 180 mgm. of nembutal was administered but made little if any grossly apparent change in spleen size. The organ was excised as before and after ashing and estimation of the radio-iron content, the volume of cells contained was found to be 15 ml. Thus these values accounted for only 2 and 5 per cent respectively of the circulating cell mass, being decidedly lower than those obtained in the other dogs.

Originally planned as controls, the same procedure was applied to the dogs after splenectomy as used in experiments 5 through 11, table 1. Dog 39–196 was given nembutal and the circulating cell mass determined. Epinephrine was injected and another red cell isotope concentration done showing that the cell mass had increased by 14 per cent. The same "control" experiment on dogs 1-G and 2-G showed increases in the cell mass of 16 per cent and 11 per cent respectively.

Where splenectomy was performed under ether the weights of the spleens in three instances were 62, 58 and 77 grams. In three succeeding splenectomies under nembutal the weights of the removed spleens were 211, 235 and 228 grams.

Discussion. In 1936 Essex, Seeley, Higgins and Mann (4) reported that ether anesthesia caused a marked increase in the erythrocyte count, hemoglobin concentration, and the venous hematocrit value in dogs, which effect they ascribed to a profound constriction of the spleen. On the other hand they found that sodium amytal anesthesia caused a drop in the red cell count which they felt was due to the removal of a considerable percentage of the circulating red cells by dilatation of the spleen.

At about the same time Seeley, Essex and Mann (11) reported that sodium amytal alone, or preliminary to ether anesthesia, resulted in a marked delay in the onset of shock produced by intestinal manipulation, as compared to the results with ether alone. Following up these findings Kendrick and Uihlen (10) recently showed that splenectomised animals went into shock more rapidly than the non-splenectomised animals, indicating that the spleen aided the or-

ganism in resisting shock. However with or without spleens shock onset was slower when ether anesthesia was supplemented by nembutal than when ether was used alone.

The experiments in table 1 indicate that under the influence of nembutal there may be a considerable number of red cells sequestered from the active circulation. This is in contrast with what is found in the normal or anemic unanesthetised animal (6). In the first set of experiments (1 through 4), table 1, in which the spleen was excised and the contained red cells measured directly it is apparent that this organ can and does retain a large fraction of the red cells under the influence of this drug. The next set of observations (5 through 11) also indicate that the drug causes the removal of large numbers of cells from the active circulation, but this approach is not specific and fails to show where the cells are pooled. The fact that there may still be about 15 per cent of the cells sequestered in the splenectomised animal under nembutal (12 through 14), table 1, shows that the spleen is not entirely responsible for this reaction, or at least that in the absence of this organ other viscera or the vascular system itself may perform this function. In a larger series it might be seen whether on the average the first and third types of reactions would in their summation give values of the order of the second or less specific group.

In the intact, nembutalised dog there is a marked increase in the venous hematocrit value following the administration of epinephrine as can be seen in experiments 5 through 11, table 1. A similar though less marked increase accompanies the injection of the drug in the unanesthetised dog (2) (3) (6) but under the latter conditions there has not been demonstrated a concomitant increase in the circulating cell mass (6). In the unanesthetised dog following splenectomy the hematocrit response to adrenalin is absent. In the nembutalised dog the reaction is much smaller in magnitude after splenectomy than before but it is not entirely abolished. These findings would indicate that in the normal unanesthetised dog the spleen either contains no appreciable pool of red cells, or that the circulation of blood through the spleen is about as rapid as in the rest of the vascular system. The effect of nembutal would seem to be the sidetracking of a large fraction of the circulating cells, partly by the spleen and partly by other tissues. This nembutal effect is temporarily reversed by the administration of epinephrine.

It is felt that the above experiments may be useful in interpretation of the delayed onset of traumatic shock following the administration of nembutal.

SUMMARY

When the circulating red blood cells of the dog are tagged with other dog cells containing the radioactive isotope of iron, and nembutal anesthesia is induced, removal of the engorged spleen shows that up to 30 per cent of the circulating red cells may be present in this organ as shown by the radioactivity of the contained cells.

When red cells have been sequestered from the circulation by the influence of nembutal, the actively circulating cell mass may be determined by the tagged-

donor-cell technique. Administration of epinephrine by vein results in an increase in the actively circulating cells as shown by dilution of the tagged cells. This increment in circulating cells has been found to be as much as 37 per cent of the total cell mass.

When the latter procedure is applied to splenectomized animals, there is still a marked response to epinephrine, an increment of red cells being added to the circulation. The increased mass of circulating cells under these conditions is about half as great as that obtained in the intact dog.

The spleens removed under nembutal anesthesia were about four times the weight of the organs taken out under ether.

The possible relationship between these findings and the observed delay of onset of traumatic shock following administration of nembutal is suggested.

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QUANTITATIVE MEASUREMENTS OF CEREBRAL BLOOD FLOW IN THE MACACQUE MONKEY[1]

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After reviewing the evidence available in 1936, Wolff (12) made the following statement: "Unfortunately, the amount of blood going to the brain still remains an uncertain quantity." As far as we know nothing has happened subsequently to remedy this situation. In the present paper we report experiments in which cerebral blood flow has been measured quantitatively under conditions which, although not strictly normal, were considerably less abnormal than those existing in perfusions of excised brains—the only circumstances under which comparable measurements have hitherto been made.

The voluminous literature on the physiology and pharmacology of the cerebral circulation has been reviewed recently (1, 4, 12) and need not be discussed here. Reasons for our own interest in these problems, the various methods we have used to study them, and the results obtained, have been presented in a series of publications from this laboratory (4, 5, 6, 7, 8, 9). The latest of these (4) contained an elaboration of the theme of the first (6) in regard to the anatomical and instrumental difficulties involved in quantitative measurements of cerebral blood flow. At that time we hoped, by appropriate modifications, to adapt a thermostromuhr to the purpose. Our misgivings (4, p. 255) regarding the reliability of that instrument for quantitative purposes were proved by our subsequent experience to be well founded, and the recent careful studies of Gregg and his collaborators (2) have amply confirmed them.

In the present experiments we have measured cerebral arterial inflow directly by a method first employed to secure the *in vivo* calibrations of the thermostromuhr already referred to (4, pp. 255 and 263) and by so doing have finally obviated the instrumental difficulties. The anatomical difficulties (4, 6) have been circumvented partly by the use of the monkey (macacus rhesus), in which, as in man, there are only insignificant communications between the intracranial and extracranial parts of the cephalic circulation (1), partly by ligation of the basilar artery, a procedure which not only forces all of the blood entering the brain to pass through the measuring device, but also prevents escape of some of it into extracranial tissues through muscular branches of the vertebral arteries (4, pp. 237 and 254).

METHOD. The measuring device is a refinement of the "simple flowmeter" described by Soskin, Priest and Schutz (10). Several models have been tested but that shown in figure 1 has proved most satisfactory. It is provided with a

[1] This investigation was largely financed through the National Committee for Mental Hygiene from funds granted by the Committee on Research in Dementia Precox founded by the Supreme Council, 33° Scottish Rite, Northern Masonic Jurisdiction, U. S. A.

jacket through which water at a temperature of 38 to 40° is circulated from a thermostatically controlled bath. The blood, rendered incoagulable by heparin, passes through the meter on its way to the brain and the volume of flow is measured by timing the passage of an injected air bubble (about 0.2 cc. in our meters) over a space of known volume (about 6 cc. in most of these experiments). The air bubble is removed by a suitable trap before the blood reënters the arterial circulation and the measurements involve no interference with or alteration in the actual blood flow.

Male monkeys weighing 3 to 6 kilos were used. They were anesthetized with nembutal (about 0.04 gram per kilo intraperitoneally). A tracheal cannula was inserted, blood pressure was recorded by a mercury manometer from a femoral artery with heparin-saline as the anti-coagulant, respiration was registered by a conventional pneumograph-tambour system, and intravenous injections were made through a burette-cannula system connected with a femoral

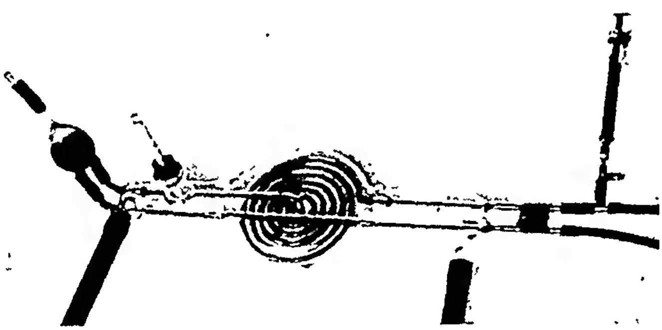

Fig. 1. Bubble flowmeter. A tuberculin syringe is connected through a small Bunsen valve system to a 22 gauge stainless steel tube so that bubbles, each of about 0.2 cc., can be injected into the inflowing stream. The space between the marks on the tube represents about 6.0 cc. The trap for collecting air bubbles is at the left. The large rubber tubes in the foreground connect the water jacket to a thermostatically controlled water bath.

vein. The operation for insertion of the flowmeter involved reflection upward of the larynx and esophagus, exposure of the anterior inferior aspect of the occipital bone, perforation of it by means of a dental burr, opening the dura, and passing a ligature around the basilar artery so that it could subsequently be tied. Silver clips, though easier to apply, turned out to be less dependable than actual ligation. Both common carotids were exposed to a point beyond the bifurcation and both external carotids were tied near their origins. Heparin was given intravenously as an initial 1000 unit (1 cc.) dose followed by 200 units every 15 minutes. A short glass cannula was inserted in each common carotid to collect blood coming from the heart, and these two were connected through a T-tube to the inflow side of the meter. The outflow tube was connected to a similar pair of cannulae inserted more peripherally in the common carotids with their tips pointing cephalad. The location of the needle for injecting the air bubbles is indicated in figure 1. In 6 experiments we attempted to connect a smaller (2 cc.) meter to the cephalic end of the basilar artery, so that flow could be measured in the carotid and basilar systems simultaneously. We succeeded in 3 of these

attempts but in the others the basilar artery was too small or too short for cannulization and we had to be satisfied with measurement of carotid flow with the basilar tied. At the end of each experiment a dye (usually india ink) was injected through the meter and its distribution ascertained by dissection. The only communications with tissues outside the brain were occasional small anastomoses around the basilar ligature, leading to spots of dye in the muscles of the neck, and occasional twigs in the orbits; these channels were so small as to be negligible, we believe. The brain was removed at the end of each experiment and its weight anterior to the basilar ligature was determined.

We have subjected this flowmeter to extensive calibration tests with a perfusion pump, and have also tried it in a number of pilot experiments on dogs and cats. As a result we are satisfied that it is dependable and accurate. There is a tendency toward overestimation of the actual flow, and the magnitude of this error increases both with the volume of the flow and with the viscosity of the fluid, but the deviation is almost imperceptible at flows smaller than 50 cc. per minute and even at the largest flow encountered in our *in vivo* measurements (103 cc. per minute) it would amount to less than 10 per cent. A correction could easily be applied for this but we have not thought it desirable to do so because of the implication of quantitative precision which the operative and other deviations from normal make illusory. The viscosity factor is much smaller than in the venturi meter (11) or rotameter (2). With these, even approximate estimations of flow call for accurate measurements of viscosity, not only for each experiment but for every supposed change in flow. With the bubble flowmeter even the change in viscosity from saline to whole blood has a barely measurable influence at flows lower than 50 cc. per minute; above that level the divergence increases progressively but it is only about 5 per cent at 100 cc. per minute. This independence of viscosity changes is the greatest advantage of the bubble flowmeter over the rotameter.

So far we have successfully employed this method to measure cerebral blood flow in 19 monkeys. The average weight of these animals was 4.2 kilos, the extremes being 3 and 6.2. The average weight of the brain above the level of the basilar ligature was 91 grams and the extremes were 85 and 105.

1. *The volume and range of cerebral blood flow.* The averages of our findings are shown in table 1. The "normal" values are those recorded at the start of each experiment. In many cases blood pressure fell considerably during the final stages of the preparation and in these pressure was restored, by intravenous injection of blood saved from an earlier experiment, approximately to its initial level before the "normal" readings were obtained. The "maximum" and "minimum" figures are derived from the highest and lowest flows recorded in each experiment, terminal states of progressive circulatory failure being excluded. The complete data are omitted in the interests of space conservation. A brief description of their distribution is therefore desirable.

In the 19 experiments in which flow was measured only through the internal carotids the "normal" flows ranged from 27 cc. (0.27 cc. per gram) to 78 cc. (0.81 cc. per gram); the corresponding blood pressure readings were 70 and 140

mm. Hg. The "maximum" flows in 7 instances were measured after intra-arterial injection (see below) of aminophylline or caffeine while blood pressure was lower than it was at the time of the "normal" reading; a similar coincidence was encountered in one experiment during a metrazol convulsion and in another during inhalation of oxygen. In the remaining cases the maximum flow was associated with a rise in blood pressure and was produced by intravenous injection of adrenalin in 4, by inhalation of oxygen in 2, and by inhalation of nitrogen in one, while in 3 it was encountered during the "normal" period. The highest flow (103 cc. total, 1.13 cc. per gram) was associated with a drop of blood pressure from a "normal" of 170 to 162 mm. during inhalation of oxygen; the "normal" flow was 75 cc. (0.77 cc. per gram). The "minimum" flow readings in 13 cases corresponded with a fall in blood pressure. Of the 6 in which at the time of the minimum flow pressure either was unchanged or elevated as compared with the "normal," 2 were obtained after intra-arterial injection of adrenalin and 2 after similar injection of benzedrine, one was found after intravenous in-

TABLE 1

	Cerebral flow		B-P	
	cc./min.	*cc./g./min.*		
Average normal	55	0.60	125	19 expts.—carotid flow, basilar tied
Average maximum	69	0.76	131	
Average minimum	25	0.27	109	
Average normal	60	0.63	97	3 expts.—carotid plus basilar flow
Average maximum	77	0.81	131	
Average minimum	42	0.44	86	
Average normal	42	0.45	99	Same 3 expts.—carotid flow only

jection of adrenalin, and one coincided with inhalation of nitrogen. The minimum readings ranged from 13 cc. (0.14 cc. per gram) at a blood pressure of 90 mm. following adrenalin intra-arterially, to 46 cc. (0.5 cc. per gram) with a blood pressure of 88 mm. after nitroglycerine intravenously. In the former experiment the "normal" and "maximum" values were 35 cc. (0.41 cc. per gram) at 104 mm. and 56 cc. (0.66 cc. per gram) at 100 mm.; in the latter the corresponding figures were 73 cc. (0.83 cc. per gram) at 150 mm. and 100 cc. (1.14 cc. per gram) at 152 mm.

The 3 experiments in which flow was measured simultaneously in the carotids and basilar are treated separately because they show that the figures obtained by measuring only the internal carotid streams represent about 70 per cent of the total carried by both carotids and basilar when both are open. The individual figures were 81 per cent, 67 per cent and 63 per cent. The number of observations is small but the existence of this discrepancy as well as its approximate magnitude seem to us to be clearly indicated. If the 70 per cent factor is used to correct the findings in the 19 experiments in which only carotid flow was

measured the average "normal" cerebral blood flow in the monkey becomes 0.86 cc. per gram per minute. This figure we believe to be eloser to the actual value than any that has previously been obtained.

The individual "normal," "maximum," and "minimum" figures show that while eerebral blood flow tends to vary direetly with the blood pressure, it can also undergo independent variations of eonsiderable size, particularly under the influenee of drugs. Since this eonfirms in another animal and by another method the eonclusions derived from previous experiments made in this laboratory (4, 5, 7, 8, 9) we attempted to parallel the earlier studies as far as possible.

2. *Effect of stimulation of the cervical sympathetic nerve.* Satisfactory tests were made 7 times on 4 animals and the results are summarized in table 2. A number of other trials in which mydriasis failed to occur (indicating ineffectiveness of stimulation) or measurements were unsatisfactory, are not included.

TABLE 2

Effects of cervical sympathetic stimulation

EXPT.	STIM.*		B-P		FLOW	
	On	For	From	To	From	To
					cc./min.	cc./min.
2-22	R	2′	108	100	39	38
	L	2½′	110	114	42	44
2-24	R	2′	142	142	46	57
	R	2′	140	142	44	46
	L	2′	146	138	50	34
2-26	L	2′	90	80	38	32
6-19	R	3½′	52	52	41	39

* Mydriasis occurred on the stimulated side in every case. In the experiment of 2-22 salivation also was observed.

The results give no indieation of a direet effect of any importance, in which they confirm the outcome of our first attempt (6) at measuring total eerebral blood flow. They are very different from those obtained by a thermocouple in the parietal cortex of the cat (4, 9) where cervical sympathetie stimulation regularly gave rise to an indieated deerease in blood flow, but similar to those obtained by the same thermoeouple in the medulla of the cat (5), where no such ehanges were seen. The possibility that reapportionment of the total stream may take place within the brain when the sympathetie nerve is stimulated receives some support from observations made in the last experiment shown in table 2, which is the only one in whieh we have as yet been able to study this question by separate measurement of the earotid and basilar blood flow. Of the total flow before stimulation (41 cc. per minute), 28 ec. was earried by the carotids, 13 ee. by the basilar. The earotid flow measurements during the stimulation were 27, 26, and 25 ce. The eorresponding figures for basilar flow are

13.4, 13.7 and 14 cc. The blood pressure was constant throughout. The change, though slight, is in the direction demanded by the above hypothesis. That the cerebral blood vessels of these animals were capable of constricting is shown by the results obtained with adrenalin and other sympathomimetic agents (fig. 2).

3. *Effect of changes in the blood gases.* Anoxemia was induced by inhalation of 90 or 100 per cent nitrogen. Oxygen (100 per cent) and carbon dioxide (10

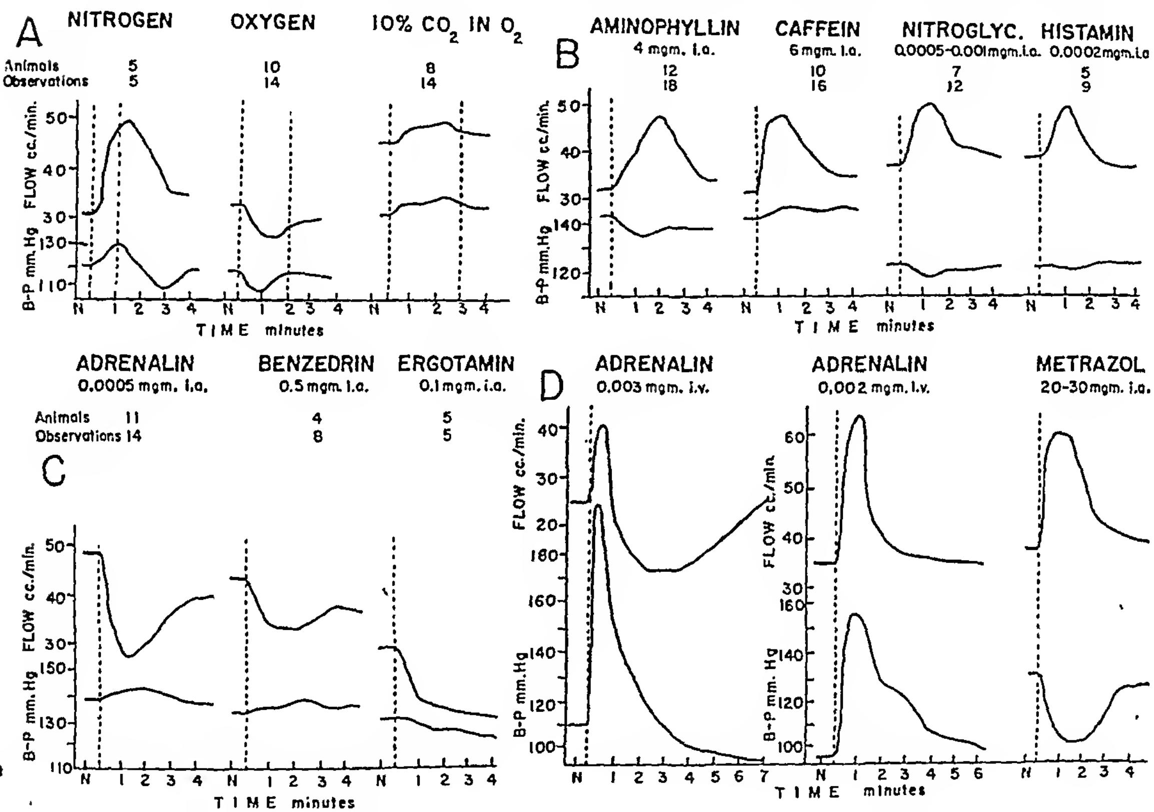

Fig. 2. Average effects on cerebral blood flow (upper) and blood pressure (lower); the numbers of animals and of observations on which curves are based are indicated. *N* signifies the "normal" value and the vertical broken line the time of injection; in A the two broken lines indicate inhalation of pure nitrogen, oxygen, or 10 per cent CO_2 in oxygen. In D the adrenalin curves represent single experiments but the metrazol record is based on the averages of the results of 8 injections in 6 animals. *i.a.* = intra-arterial injection (into the inflow tube of the meter). *i.v.* = intravenous injection.

per cent in O_2) were also tried. The average findings of these procedures are shown in figure 2 A. The individual results did not differ in any important respect from these.

The most important finding here, in our opinion, is the apparent superiority of anoxemia over hypercapnia as a dilator of cerebral vessels in the monkey. The increases in flow brought about by CO_2 were smaller than we had been led to expect by our experience with other animals, while those elicited by anoxemia were much greater. But attention must be called to the following experimental

circumstance, which may modify the deductions to be drawn from this comparison. None of these animals showed more than a slight hyperpnea during anoxemia, due, we believe, to damage to the carotid innervation during the dissection or insertion of the cannulae; that the chemoreceptor reflexes were normally active before these procedures were carried out was shown in a number of cases by vigorous responses to anoxemia or cyanide at the start of the experiment. As has been suggested elsewhere (3), the reflex hyperpnea of anoxemia may lead to sufficient reduction in arterial carbon dioxide tension to cause constriction of cerebral blood vessels. In that event the dilator effect of anoxemia would be antagonized and perhaps overcome, but in these animals no such antagonism was present. The increases in cerebral flow resulting from anoxemia may therefore have been considerably greater than under more nearly normal circumstances.

The effects of the other gases were qualitatively similar to those detected in other animals by other methods. There was a distinct hint of a constrictor action by oxygen, though this was never at all marked. The dilator action of CO_2 was consistent but not very great. In a few cases respiration ceased and asphyxia developed before oxygen could be given by tracheal catheter. In these, cerebral blood flow increased as long as blood pressure did not fall, and there was no sign of cerebral vasoconstriction associated with increased activity of the vasomotor center.

4. *Effects of drugs.* Since this preparation involved exteriorization of the arterial blood supply of the brain and therefore made intra-arterial injections very easy, we frequently availed ourselves of the advantages (see 4) of this method of studying the effects of drugs on the cerebral circulation. Data sufficient at least for tentative conclusions have been secured by intra-arterial injections of adrenalin, benzedrine, ergotamine, histamine, nitroglycerine, caffeine, theophylline, and metrazol. The results are illustrated in figure 2 B and C. They can be summarized by the statement that by intra-arterial injection of adrenalin or benzedrine the cerebral vessels can be constricted quite vigorously, and by similar injection of histamine, nitroglycerine, caffeine, and theophylline they can be dilated. Such changes can occur without any corresponding alteration in blood pressure. Ergotamine decreased cerebral blood flow, but since it also decreased blood pressure in that dosage, and smaller amounts were ineffective, the interpretation is as uncertain as was that of the results of comparable experiments on cats (4). Metrazol caused a pure and usually marked increase in cerebral flow (associated with convulsions, which came on instantly after these intra-arterial injections), while blood pressure fell sharply (fig. 2 D). Acetyl β-methylcholine (Mecholyl) was given intra-arterially 7 times in 3 animals, the dose being 0.0001 mgm. Cerebral blood flow was invariably increased while blood pressure fell slightly; recovery was complete within 3 minutes. The average figures were from 44 to 49 cc. per minute in flow, from 136 to 132 mm. Hg in pressure.

Observations on the effects of these drugs when given by channels other than the intracarotid have so far been infrequent. This is because most of the

drugs, when given in effective dosage, ordinarily have effects so prolonged that only one or two valid tests could be carried out in a single experiment. The experiments were so expensive and difficult that we tried to make as many different observations as possible in each, and intraarterial injections of minimum effective doses were preferred. However, enough intravenous injections were made of adrenalin (10 in 8 animals, excluding injections intended to restore a failing circulation) to show that the constriction of cerebral vessels which intracarotid injection of this drug produces is much less in evidence following intravenous administration (fig. 2 D). In all of these 10 instances there was an increase in flow as blood pressure rose. In 5 the flow descended faster than the pressure and reached a level lower than the starting point though pressure was at or above its control level. In the others the flow came back to a level either the same as or higher than the control. A representative example of each type of response is shown in figure 2 D.

Of the other drugs, only caffeine and theophylline have been given often enough intravenously to justify even tentative conclusions (5 times in 5 animals and 4 times in 3 animals, respectively). Caffeine, in dosage of 10 to 20 mgm., increased cerebral flow slightly in 3 cases, did not change it appreciably in the others; the most marked increase was from 24 to 29 cc. per minute; since blood pressure fell at the same time from 86 to 80 mm. this result appears to be significant. Theophylline (as the ethylene diamine derivative), when injected intravenously in dosage of 10 to 40 mgm., lowered blood pressure quite markedly; cerebral flow was decreased at the same time in 2 cases, increased in the other 2; the most marked increase in flow was from 25 to 30 cc. per minute, associated with a fall in pressure from 106 to 96 mm.—again a significant change.

We have also tried nitroglycerine and insulin by intravenous injection, each in 2 different animals. The former, in 0.5 mgm. dosage, only decreased cerebral flow as blood pressure fell and there was no sign of an effective vasodilator action in the brain. Insulin, in dosage of 5 and 10 units, was used because of the possibility that some of its convulsant effects might be due to violent constriction of cerebral vessels. No trace of any such action was evident (table 3). Blood pressure was lowered as hypoglycemia developed and cerebral flow followed this apparently quite passively. Recovery of the circulation began while the blood sugar was still falling. Convulsions did not appear, doubtless because of the anesthesia.

Posterior pituitary extract (Parke, Davis and Co. obstetrical pituitrin, 10 pressor units per cc.) was injected intra-arterially once in each of 3 experiments, the dose being 0.1 unit (0.1 cc. of a 1 to 10 dilution in saline). The result was an immediate, consistent, and considerable (though transitory) fall in blood pressure accompanied by a parallel decrease in cerebral flow. Thus in one case pressure fell from 116 to 82 mm. within 2 minutes and recovered to 120 by the end of 7 minutes. The corresponding figures for flow were 30, 12, and 28 cc. per minute. The changes in flow seemed to be the result of the fall in blood pressure, but we have not as yet investigated the latter rather surprising result any farther.

Nembutal (Abbott's veterinary solution, 6.5 per cent containing 20 per cent alcohol) was injected intra-arterially in 0.1 cc. dosage (6.5 mgm.) in 3 animals in which narcosis had become too light. The consistent result was a slight but distinct increase in cerebral flow (e.g., from 40 to 46 cc. per minute) associated with a slight fall in blood pressure (from 116 to 110 mm.), with recovery of both within 5 minutes. Other narcotics have not been tried, nor have we as yet attempted to dissociate the effect of the alcohol of this solution from that of the barbiturate.

5. *The basilar-carotid anastomosis.* In the 3 experiments (see table 1) in which we were able to measure flow through the basilar and the internal carotid systems separately, we had an opportunity to determine not only the portion of the total flow carried by each of these, but also the extent to which readjustments can occur under different circumstances. One of these findings, indicating that the carotid component amounts only to about 70 per cent of the actual total when the basilar is closed, has already been mentioned (p. 424). Other observations bearing on this subject are shown in table 4.

TABLE 3

	Animal 1			Animal 2			Comment
	Cereb. flow	B-P	Blood sugar	Cereb. flow	B-P	Blood sugar	
	cc./min.	*mm. Hg*	*mgm. %*	*cc./min.*	*mm. Hg*	*mgm. %*	
Control	51	118	109	42	108	89	
15 min. after insulin*	33	76	71	23	54	55	Hyperpnea in both animals at this time
30 min. after insulin*	28	60	61	39	100		Hyperpnea ended in both at this time
45 min. after insulin*	36	90		35	94	46	
60 min. after insulin*	39	102	43	31	115	42	Glucose in animal 2 at 65 min.**
75 min. after insulin*	43	108		29	114		Glucose in animal 1 at 80 min.**
100 min. after insulin*	35	100	54	27	112	70	

* Insulin was injected intravenously in dosage of 5 units in animal 1, 10 units in animal 2. Weight of animal 1, 5.5 kilos; of animal 2, 6.2 kilos.

** Glucose was injected intraperitoneally, 25 cc. of 5 per cent solution being given to each animal.

These data indicate several points of some importance. One is the variability of the basilar:carotid ratio. In the last experiment cited the basilar was larger than in the other two and it is probable that the ratio here (80 per cent) is quite exceptional. Furthermore, the basilar arteries in all three of these animals were larger than they were in three others in which the vessel was too small for cannulization. For these reasons we do not think it advisable to venture, from the data now available, any deduction other than that the basilar contributes a highly variable portion of the total cerebral flow. More definite conclusions can however be derived on another point, viz., the extent to which the flow

through each system is increased when the other is closed. The increase in basilar flow resulting from carotid occlusion was much greater, in two of the three experiments, than the increase in carotid flow resulting from basilar occlusion. This is to be expected in view of the relative sizes of the two sets of vessels and the volumes carried by them. It is noteworthy in this connection, however, that even the greatest percentile increase in basilar flow (90 per cent) meant that when the carotids were closed the total cerebral flow was 21 cc. per minute, which was only 40 per cent of the amount previously carried by both systems (53 cc.). In the same animal the carotids carried 43 cc. when the basilar was closed, and this, although it was an increase only of 10 per cent in carotid flow, nevertheless amounted to 81 per cent of the previous total.

TABLE 4

EXPT.	FLOWS—BOTH OPEN				CAROTID FLOW ON BASILAR OCCLUSION				BASILAR FLOW ON CAROTID OCCLUSION				CONDITIONS
	Car.	Bas.	B-P	Bas./Car.	From	To	Incr.	B-P	From	To	Incr.	B-P	
	cc./min.	*cc./min.*		*per cent*	*cc./min.*	*cc./min.*	*per cent*		*cc./min.*	*cc./min.*	*per cent*		
6-19	42	11	96	26	39	43	10	84	11	21	90	98	Control—O_2 by tracheal catheter
	37	11	80	30	39	43	10	82	11	18	64	82	Same
	28	13	48	46	28	33	18	52	13	17	30	52	After caffeine, theophylline, and nitroglycerine i.v.
6-23	54	23	140	43	26	33	27	100	23	35	52	152	Control (basilar occlusion later than others)
6-24	24	19	70	80	24	27	13	71	19	22	16	72	Control—O_2 by tracheal catheter
	22	18	72	82	24	29	21	73	18	22	22	74	After adrenalin and caffeine i.v.
	27	17	69	63	23	27	17	67					Later—follows caffeine i.a.

At the other extreme, the smallest percentile increase in basilar flow on carotid occlusion (16 per cent) brought the total flow to 22 as compared with 43 cc. or 51 per cent, and the corresponding increase in carotid flow on basilar occlusion (to 27 cc.) amounted to 65 per cent of the original total. These figures serve to show the order of magnitude of the readjustments brought about through the circle of Willis in these particular animals. The individual variations were so great as to suggest that the consequences of occlusion of these vessels in any given subject can scarcely be predicted.

SUMMARY AND CONCLUSIONS

The volume of blood flowing into the brain through the internal carotids has been measured in 19 monkeys anesthetized with nembutal. The average figure is 0.60 cc. per gram per minute at an average blood pressure of 125 mm. Hg.

Direct measurements of flow through the internal carotids and the basilar indicate that this figure probably represents only about 70 per cent of the total normal flow. The corrected value therefore is 0.86 cc. per gram per minute.

Stimulation of the cervical sympathetic nerve produced no significant alterations in cerebral flow, though in one experiment results suggesting a redistribution of blood were obtained.

Anoxemia and hypercapnia both increased cerebral flow and increased oxygen tended to decrease it. The effects of anoxemia were more striking than those of CO_2 and much more marked than was expected from comparable experiments on other animals.

Given by intracarotid injection and in minimum effective dosage, adrenalin and benzedrine produced consistent and rather striking decreases in cerebral flow and caffeine, theophylline, histamine, and mecholyl produced comparable increases. Ergotamine and pituitary extract caused a decrease in both flow and blood pressure when similarly given.

When given intravenously adrenalin increased cerebral flow as blood pressure rose; when pressure fell flow fell at the same rate in half the cases, at a faster rate (leading to a definitely subnormal flow) in the others. Caffeine and theophylline, similarly administered, sometimes increased flow distinctly, although blood pressure fell, but nitroglycerine had no such effect.

In 3 experiments in which flow through the basilar and internal carotid systems was measured simultaneously, the part contributed by each to the total flow varied widely (basilar: carotid ratio 26, 43 and 80 per cent). Flow through each system increased when the other was closed but the magnitude of the readjustments thus brought about was subject to such great individual variations as to suggest that the quantitative consequences of occlusion of any of these vessels cannot be predicted.

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THE CYTOLYTIC EFFECT OF SAPONIN ON THE WALLS OF VESSELS[1]

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In a study of the activity of hemolysins *in vivo*, Ponder, Hyman and White (1941) found that lysins such as saponin, when perfused through an organ, disappear from the perfusion fluid, presumably as a result of combining, as cytolysins, with the structures lining the vessel walls and with other tissue cells. The purpose of this paper is to relate the quantity of lysin taken up with the cytolytic effects produced, these being measured by the rate of edema, the diffusion of hemoglobin through the injured vessel walls into the intercellular spaces, and other indications of alteration in the permeability of the vessels.

1. *Edema formation.* The effect of lysins on the rate of development of edema was determined by the method of Hyman and Chambers (1942). Essentially, the method involves the perfusion of frog legs (Rana pipiens) through a cannula inserted into the lower end of the aorta, the perfusion fluid passing into the vascular system of the muscles and skin, and out through the renal portal system and kidneys, to escape through the cut end of the vena cava. A mixture of 1.5 per cent CO_2 and 98.5 per cent O_2 under pressure was used to drive the fluid from the storage reservoirs and to aerate the solutions properly. The perfusion pressure was maintained at 20 mm. Hg by means of a mercury blow-off. The cannulated preparation is placed in a light cylindrical moist chamber suspended by a helical phosphor-bronze spring. As the legs become more and more edematous during a perfusion, their increased weight causes an extension of the spring, and this is continuously recorded by means of a lever writing on a slowly moving drum. The spring should be sufficiently sensitive to record the passage of single drops as they fall from the preparation, thus acting as a drop recorder; in this way changes in the vascular caliber as well as in edema rate can be observed.

The perfusion fluid used was a 0.5 per cent solution of Eastman Purified Calfskin Gelatin in frog Ringer buffered at 7.65.[2] The lysins were prepared in various concentrations in this fluid. In determining the effect of any concentration of saponin we proceeded as follows: The preparation was perfused with the perfusion fluid for about 1 hour in order to find the rate of edema formation with this fluid alone. During this period the weight curve presents a constant slope. The solution containing saponin was then started through the cannula, the commencement of the perfusion with lysin being signalled by mixing a small

[1] This work was carried out with the aid of a grant to one of us (E.P.) from the American Association for the Advancement of Science.

[2] This concentration of gelatin has a lower colloid osmotic pressure than that of frog plasma, and was used in order to assure a measurable edema rate during the initial period of perfusion with the fluid alone.

amount of a non-toxic dye with the first small volume of the fluid containing lysin, and observing the time of appearance of the first colored drop. For the first few minutes of the perfusion with lysin, the slope of the weight curve remains the same as before, but at the end of this latent period it begins to increase, at first slowly and then more rapidly. This is an indication of an increasing permeability of the vessel walls. After attaining a maximum slope, the weight curve begins to flatten out; this is partly the result of a vasoconstriction produced by the saponin, as shown by the relative infrequency of the outflowing drops. In this investigation we are concerned with the weight curve only up to the point of its maximum slope.

The rate of increase in weight of the preparation, i.e., the rate of edema formation, is a measure of the permeability of the vessel walls, since it represents the transfer of a quantity of fluid dQ in time dt. The permeability in the absence of lysin is accordingly measured by the constant slope of the weight curve during the first hour of perfusion. Similarly, the permeability at any time after the introduction of the lysin is measured by the slope of the curve at that time, and the effect of the lysin on the permeability is conveniently measured by the ratio r of that slope to the constant slope obtained during the preliminary perfusion. Making the same kind of assumptions as made for the action of lysins on red cells (Ponder, 1941), let us think of the permeability in the absence of the lysin as involving the passage of fluid through pores occupying a total surface A_1 of the vessel wall, and of the lysin as increasing the number or size of the pores so that the additional surface A_2 is involved. A_2 will then be proportional to $(r - 1)$, which again will be proportional to the amount of lysin used up in breaking down or otherwise transforming the surface of the vessel walls to form the new pores. Further $(r - 1)$ should be some function of c_o, the concentration of lysin in the perfusion fluid, and the kind of function which it turns out to be experimentally is shown in table 1 and in figure 1, in which the maximum value of $(r - 1)$ found for each concentration is plotted against c_o. Each value is the average of from 3 to 8 individual determinations. Similar increases in edema rate have been obtained with sodium taurocholate (1 in 2,500), but, as is well known, this lysin is not so suitable for quantitative work as is saponin.

Figure 1 shows that the relation of $(r - 1)$ to c_o is substantially linear, the intercept on the ordinate, $c_o = 8.5\ \gamma/\text{ml.}$, corresponding to a quantity of lysin just too small to produce a change in permeability. This quantity measures the lower limit of the frequency distribution of resistances in the vessel walls to breakdown by the lysin (cf. Ponder, 1941, fig. 3). The linearity of the relation shows that both A_2, the area transformed so as to become permeable, and the amount of lysin used up in the transformation, are proportional to c_o, a result arrived at independently below, and familiar because it is met with in other related systems, such as those in which saponin produces cytolysis of white cells (Ponder and Macleod, 1936) or enters into combination with red cell ghosts (Ponder, 1935).

We expected to find the latent period, i.e., the time between the arrival of the lysin at the preparation and the time at which the first increase in edema rate

was observed, to be smaller for high concentrations than for low ones. This may indeed be so, but the experiments do not show it, probably because the con-

TABLE 1

DILUTION 1 IN	c_0	$(r - 1)$	SAPONIN SPACE	U
	$\gamma/ml.$			γ
20,000	50	5.0	5.3	1900
35,000	29	2.5	3.5	1220
40,000	25	1.8	2.9	980
50,000	20	1.5	2.5	760
60,000	17	1.1	1.9	620
100,000	10	0.2	1.0	350

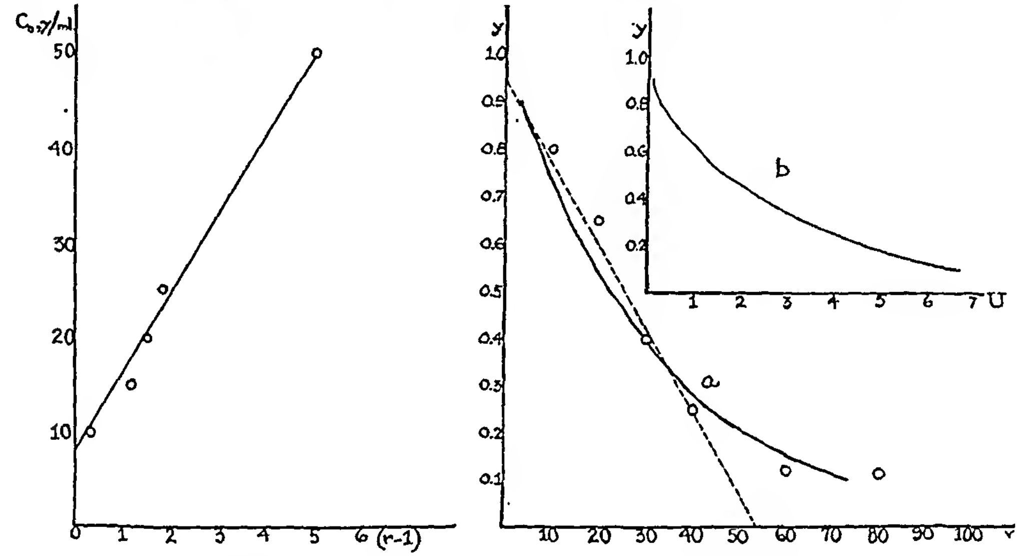

Fig. 1 Fig. 2

Fig. 1. Increase of edema rate (abscissa) plotted against concentration of saponin in perfusing fluid (ordinate) for perfusions of 30 minutes' duration.

Fig. 2. Curve a, experimental curve for fraction of lysin taken up, y, plotted against v, the volume of perfusion fluid passed through. Dotted line, Ponder, Hyman and White's approximation. Curve b the integral of curve a (equation 4).

centration range is relatively small and the experiments show so much individual variation.[3] We also expected to find the maximum increase in weight after

[3] The variability of the material in these experiments is considerable, and we have been unable to eliminate it. An average value of r, such as the 2.5 given for 20 γ/ml., for example, is subject to a variation of ± 1.0, and occasionally experiments have had to be rejected altogether because of the extreme vasoconstriction produced by the lysin. The variability in the diffusion of hemoglobin experiments is smaller, amounting to ± 10 per cent, but about one out of every four experiments had to be rejected for technical reasons, the principal ones being unequal perfusion rates in the two legs and the appearance of extreme vasoconstriction. The experiments on the uptake of lysin are the best of all, and the great variations in the perfusion rate found by Ponder, Hyman and White in their kidney perfusions were almost completely absent. R. pipiens is better material for all these types of experiment than R. catesbiana.

perfusion with the lysin to be greater in high lysin concentrations than in low ones. The experiments show almost the reverse, the average increase in weight from the beginning of the perfusion with saponin to the maximum of the weight curve being 16, 17.8 and 26.9 per cent of the initial weight for the three concentrations 29, 20 and 17 γ/ml. This is undoubtedly due to the powerful vasoconstriction produced by the higher concentrations of lysin, this preventing a true maximum being reached.

2. *Diffusion of hemoglobin.* Danielli (1941) has described a method for measuring the volume of the vascular system of a muscle by perfusing it with a hemoglobin solution. If a lysin such as saponin is added to the perfusing fluid, cytolysis occurring in the vessel walls allows the hemoglobin to diffuse out of the vascular system into intercellular spaces and perhaps into the muscle cells themselves. The extent of this steadily increasing "saponin space" can be taken as a somewhat arbitrary measure of the amount of destruction produced by the lysin at any time during the perfusion.

The experiments were carried out along lines suggested by Danielli's paper. The hind legs of frogs (R. pipiens) weighing about 50 gram were perfused through the aorta just above the bifurcation, the perfusion being begun within 3 minutes of the pithing of the animal. The vessels were first washed out for 15 minutes with a 2 per cent gelatin in frog Ringer, and were then perfused with a 3 per cent solution of hemoglobin prepared by hemolysing sheep red cells with water, adding salts to bring the salt concentration to that of frog Ringer, centrifuging, and filtering. This perfusion was usually continued for 30 minutes. At the end of this time one common iliac artery was tied off, and the gastrocnemius of the leg on that side was used as a control. The remaining leg was perfused for another 30 minutes with a hemoglobin solution of the same concentration, but containing saponin in known amount, e.g., 20 γ/ml. or 1 in 50,000. The higher concentrations of saponin tend to produce vasoconstriction, which can be off-set to some extent by increasing the perfusion pressure, initially 20 mm. Hg. The perfusion fluids were kept continuously oxygenated.

The gastrocnemii were removed, rinsed, dried with blotting paper, and weighed. Each was then minced and extracted in 5 ml. of distilled water for 1 hour. The extracts were cleared of particles by centrifuging and, after adding an equal volume of 2 per cent HCl and allowing to stand for 30 minutes, the amount of hemoglobin present was found with a photoelectric colorimeter. A correction was made for the effect of the light-scattering substances which are extracted by this procedure from unperfused muscle, and another correction, arrived at in a similar way to that described by Danielli, was made for the incomplete recovery of the hemoglobin from the minced muscles. We have found more variation in the corrections required than Danielli reports, but experience with the method of making them has satisfied us that they are good enough for our purpose.

The results of the experiments can be summarised under 3 heads. *a.* The volume of the vascular system of the frog gastrocnemius perfused with 3 per cent hemoglobin in Ringer is 4.7 per cent of the muscle volume, with a variation

of ±0.6 per cent from frog to frog. This confirms Danielli's result. *b*. When saponin is added to the perfusion fluid, its cytolytic effects enable the hemoglobin to diffuse out of the vascular system into a steadily increasing "saponin space." Expressed as a multiple of the original volume of the vascular system, the saponin space found after a fixed time is an approximately linear function of the lysin concentration. This is shown in table 1, which gives results for perfusions of 30 minutes' duration (22 expts.). Similar results, not shown in the table, have been obtained for sodium taurocholate. *c*. As the perfusion with any one concentration continues, the saponin space increases towards a limit, no doubt related to the limit of extensibility of the tissues. At first the increase is approximately linear with time, but later on it becomes slower. The increase in the saponin space with time presumably results from the continued cytolytic action of the saponin, first on the vessel walls, enabling the hemoglobin to penetrate into extravascular spaces, and later on the boundaries of these spaces, enabling still further penetration to take place.[4]

3. *Uptake of lysin by perfused legs.* The extent to which the cytolysin saponin is taken up by the tissues during these perfusions can be found by using various concentrations for perfusion and determining by means of a hemolytic titration the concentrations which appear from time to time in the perfusates (Ponder, Hyman and White, 1941).

Weighed frog hind limbs (R. pipiens) were perfused through the aorta with saponin in dilutions of from 1 in 20,000 to 1 in 100,000 in frog Ringer. The vessels were first washed out for 15 minutes with frog Ringer, after which the saponin perfusion was begun under a pressure of 20 mm. Hg and continued for from 1 to 3 hours. Successive 5 ml. samples of the perfusate were received into vials. The time required for the collection of each was recorded, the effects of vasoconstriction being offset, so far as possible, by increasing the perfusion pressure. The concentration of saponin in each successive 5 ml. sample was subsequently determined by a hemolytic titration.

If we plot y, the fraction of the initial concentration c_o removed as a result of combination with the tissues at any time t, against v, the volume of the perfusate which has passed through, we get a curve such as that shown in figure 2, *a*. The curve, convex to the v-axis, is quite flat; the position of the points at the lower end is somewhat uncertain because of the lower precision of the hemolytic titrations in this region, and the whole shape of the curve is liable to be affected by lack of constancy in the perfusion rate.[5] But if the lysin, supplied at a con-

[4] Before Danielli's paper appeared, we had carried out a number of perfusions with the dye Evans' Blue, before and after the addition of saponin. This dye remains confined to the vascular bed in the absence of the lysin, and perfused muscle stains a pale blue. When saponin is added, the dye escapes through the cytolysed vessel walls, and the muscle becomes intensely blue. We were unable to make these experiments quantitative because we could not extract the dye from the muscle.

[5] In their experiments on the uptake of lysin by kidney tissue, Ponder, Hyman and White obtained relations similar to these, but they used a straight line with a small intercept on the y-axis as an approximation to the relation between y and v. In the light of the experiments in this paper, this is not admissible even as an approximation, because the

stant rate, combines with a component S_o (which can be put, for convenience, as = 100) of the vessel wall and transforms S of it, the rate of disappearance of lysin should be proportional to the amount of surface left untransformed, or

$$-dy/dt = -dy/dv = k(S_o - S) = k(y) \ldots\ldots\ldots\ldots (1)$$

Integrating,

$$v = 1/k \, . \log S_o/y \ldots\ldots\ldots\ldots (2)$$

which is the equation of the experimental curve obtained by plotting y against v, as in figure 2, a, where S is put = 100 for convenience, and in which k = 0.0313. If the perfusion is allowed to continue until the combining power of the tissues is exhausted, the total amount of lysin taken up will be

$$U = 1/k \int \log (S_o - y) . dy \ldots\ldots\ldots\ldots (3)$$

$$= 1/k \, \{ \log S_o - \log y - S_o/y + 1 \} \ldots\ldots\ldots (4)$$

Curve b in figure 2 shows the value of this integral for various values of y, S_o being again put = 100. In practice, U is found directly from the experimental curve (e.g., figure 2, a) by graphical methods.

From (4), if y and U are both measured in γ/ml., we should have U/c_o = a constant if S_o is constant, as it should tend to be for any one kind of material. Thus U should be proportional to c_o, and this is what is found in a series of perfusions of frog legs with saponin (table 1, last column). This means that while the uptake capacity of the reaction surface is still unexhausted, an approximately constant fraction of c_o is taken up per unit surface. Such a relation is similar to that found by Ponder and Macleod (1936) for the amount of lysin taken up by suspensions of leucocytes as a function of time and of lysin concentration: "The disappearance of lysin is rapid at first, and tends to reach a final value asymptotically (cf. fig. 2, b), the amount which finally disappears being an almost linear function of the amount initially present". Similar relations have been obtained for the uptake of saponin and other lysins by red cell ghosts (Ponder, 1935), by kidney tissue (see footnote 4), and, indirectly, in the first section of this paper. The relations, in fact, seem to be quite general.

It remains to relate the quantity of saponin which disappears in the course of a perfusion to the effect which it produces, and here we must be content with an order of magnitude only. For the concentration 20 γ/ml. the maximum edema

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point $y = 100, v = 0$ has absolute precision and must lie on the curve relating y and v. Looking back over the kidney data, it is clear that in most of the cases in which a sufficient experimental range was covered the relation between the variables is a curve convex to the v-axis. The approximation $U = c_o \cdot ab/2$, made on the basis of the linear relation, nevertheless gives U/b as a nearly constant fraction of c_o (Ponder, Hyman and White's table 2), which is analogous to what we find here. Note: The expression of y as a percentage rather than as a fraction of c_o in Ponder, Hyman and White's paper has resulted in an error in an index on p. 21. The amount of saponin combined with 1 gram of kidney tissue should be 130, and the factor of loss should be 3.4 (10^{-2}). This makes the effectiveness of a lysin *in vivo*, as compared with its effect *in vitro*, 1/2000th.

rate $r = 2.5$ is reached after about 30 minutes, and after the same time we have a saponin space of about 2.5 times the initial vascular volume of the muscle. Now for this concentration and time, $U = 420$. Taking 3μ as the radius of a capillary, which is a minimum value in the presence of a capillary poison, and assuming that the capillary bed makes up 5 per cent of the muscle volume, we find that 420 γ of saponin would form a layer on the capillary walls about 120 Å. thick, corresponding to something less than 10 saponin molecules in depth. For the changes in edema rate and in saponin space measured here, the quantities of lysin involved are accordingly of the same order, per unit area of surface, as the quantities involved in bringing about breakdown of the red cell membrane.

SUMMARY

When frog muscle is perfused with saponin or bile salts in frog Ringer, the permeability of the vessel walls is increased, and this results in an increased rate of edema formation. If the muscle is perfused with a solution of hemoglobin to which saponin or bile salts have been added, the hemoglobin, which in the absence of the lysins remains in the vessels, escapes through the vessel walls and appears in the extravascular spaces. While these effects are being produced, the lysins disappear, in part, from the perfusion fluid, being taken up by the vessel walls and other tissue cells. Quantitative determinations of the rate of uptake of the lysins and of their effects on permeability show that the kinetics of the cytolytic process are similar to the kinetics of hemolytic processes. The quantities of saponin involved in producing these changes in permeability are such as would cover the walls of the vascular system of the muscle with a layer of lysin less than 10 molecules thick.

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EFFECT OF HYPOPHYSECTOMY AND OF PURIFIED PITUITARY HORMONES ON THE LIVER ARGINASE ACTIVITY OF RATS[1]

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The rôle of the pituitary in controlling nitrogen metabolism has long been recognized. Thus the growth stimulating action of pituitary extracts was shown to be associated with nitrogen retention, while the rate of protein breakdown appears to depend upon the adrenocorticotropic action of the hypophysis. Both the protein anabolic action of growth hormone and the catabolic action of adrenocorticotropic hormone (ACTH) influence the rate of urea excretion and it therefore appeared indicated to investigate the effect of various hormones on the enzyme systems involved in the formation of urea. Arginase was chosen as the first enzyme to be studied, since it seemed to be more specifically connected with the formation of urea than amino-acid oxidases, transaminases, etc. Even if the Krebs cycle for the formation of urea should not withstand criticism without modification, the involvement of arginine and arginase in the formation of urea appears well established.

Studies of the hormonal control of liver arginase were initiated by Lightbody and Kleinman (1) who investigated the action of thyroxin on this enzyme system. In view of the fact that thyroxin may show variable effects in regard to nitrogen metabolism, dependent on the dose and other endocrine factors, its lack of a clear-cut action on the arginase activity is not too surprising. It was hoped that certain pituitary factors might reflect their unequivocal action on nitrogen metabolism in more consistent changes in liver arginase concentration. This appeared to be the case when the adrenocorticotropic hormone (ACTH) was found to increase liver arginase activity markedly (2). Confirmation of this finding, which has recently been extended to certain adrenal steroids (3), will here be presented. Besides, an action opposite to that of ACTH will be demonstrated to be exerted by other pituitary hormones, primarily the growth hormone. Furthermore, the effect of hypophysectomy which was found to lead to a decrease in the arginase activity of rat's livers, will be discussed.

Experimental conditions, methods and materials. Rats of the Long-Evans strain of both sexes and various age groups were used for these studies. For most experiments immature females were employed, many of which were hypophysectomized at the age of 26 to 28 days. For another series of experiments, 2 to 3 month old males were used, either from the day following the operation, or 2 to 4 weeks postoperative. They were treated with hormone solutions

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for various time periods, ranging from 4 hours to 15 days, receiving one or two intraperitoneal injections daily. In many experiments three injections were given during the 24 hours preceding autopsy. All rats were fasted, either for 24 hours or 7 to 8 hours, before autopsy. Since the arginase content of untreated rats was found to differ for the two fasting periods, the data were grouped according to the length of the fast. Preceding the fast the animals were fed ad libitum, with the exception of a few experiments in which both hypophysectomized rats and their unoperated controls were fed equal amounts of diet by stomach tube.[2]

For the determination of arginase, the livers were removed under sodium amytal anesthesia and immediately weighed and placed on ice. In the case of hypophysectomized rats, the sella was then searched for residual pituitary tissue and only the livers of completely hypophysectomized rats were used. In a number of experiments, individual livers were analysed, but in general about one-third of each liver was cut off, these fractions then being pooled according to groups and analyses of the pooled livers performed in duplicate. For that purpose the liver tissue is broken up and finely suspended in a hundred fold of distilled water by means of a Waring Blendor, 1 or 2 minutes being generally used, at the low speed. The suspensions are then centrifuged and 0.5 cc. of this liver extract added to 1 cc. 2.5 percent arginine carbonate solution and 1.25 cc. 0.1 M pH 9.5 glycine sodium hydroxide buffer. Then 1 cc. water is added and the tubes (pyrex test tubes, graduated at 5 cc.) placed in a water-bath at 38 to 39°C. for 60 minutes. They are then immediately transferred into a boiling waterbath for 15 minutes, to inactivate the enzyme. The amount of urea formed by the enzyme action is determined by the use of xanthydrol. For this purpose the digests are made up to the 5 cc. mark and 2 cc. is pipetted into 15 cc. centrifuge tubes. To this is added 7 cc. glacial acetic acid and 0.2 cc. 10 percent methylalcoholic xanthydrol solution 5 times at 10 minute intervals. The solutions are occasionally stirred with glass rods during this time and again after a few hours standing, to ensure complete crystallization of the urea-xanthydrol condensation product. The following day these precipitates are centrifuged off, washed with 3 cc. methanol, again centrifuged and drained. They are then redissolved by the addition of concentrated sulfuric acid, quantitatively washed into micro-Kjeldahl flasks and their nitrogen determined. The unitage of the enzyme concentration per gram liver is calculated from these urea-nitrogen values according to Edlbacher (5). Since activators are neither added nor removed from the liver extracts, the determination gives a measure of arginase activity rather than of the concentration of the enzyme in these livers. Whenever in this discussion arginase content or concentration is mentioned, reference is made to the naturally activated arginase or the arginase activity per gram liver tissue.

A great number of duplicate digests with the same liver extract, as well as duplicate urea determinations after digestion gave values which generally were within 2 to 3 percent, differing rarely by as much as 10 percent. If the liver

[2] A diet modified by W. Marx (4) to assure easy passage through a narrow catheter tubing.

extracts were kept at room temperature for a few hours before use, or were stored at 2 to 5°C. for 2 days, their arginase activity was found 10 to 20 percent lower than in the fresh tissue. Therefore all analyses were generally performed immediately following autopsy.

Through analyses of individual livers of similarly treated rats a measure of the variations that are to be expected within groups was obtained. Statistical analysis of these results indicated that in groups of 4 rats a positive difference of 33 percent or a negative one of 25 percent was significant ($P<0.05$) whereas differences smaller than 20 percent were not significant in groups of this size. Assuming variations to be similar in experiments where they were not determined owing to analyses being performed on the pooled livers, differences exceeding 20 percent always led to a repetition of the experiment and final conclusions were only drawn when such or greater differences were obtained in a series of experiments.

The hormone preparations were the same as those used in other recent metabolic studies (6), part of the data being obtained from the same rats. The adrenocorticotropic (ACTH), growth, lactogenic and interstitial cell stimulating (ICSH) hormones contained probably no more than one percent of any one of the other hormones, while thyrotropic and follicle stimulating fractions may have been contaminated by 10 to 15 percent of ICSH. The authors are indebted to C. H. Li, W. Marx, W. R. Lyons and J. Fraenkel-Conrat for kindly supplying many of these preparations.

Results. *Effect of hypophysectomy on liver arginase.* A comparison of the arginase activity of the livers of similar untreated normal and hypophysectomized rats indicated a great difference, the enzyme concentration in normal rats being about twice as high as in the operated rats (table 1). In view of the well established dependence of arginase concentration upon diet and food intake (7), it appeared essential that this factor be carefully controlled before the difference between normal and hypophysectomized rats could be attributed to a specific action of the pituitary. To that end part of a group of immature female rats was hypophysectomized; both operated and normal control rats were then fasted for 24 hours; for the ensuing five days they received as much diet as was voluntarily consumed by the hypophysectomized rats and the next four days all were fed equal amounts by stomach tube, followed by a 24 hour fast preceding autopsy. Also under these conditions, the operated rats showed arginase activities which were exactly half of those of the normal rats (average of four hypophysectomized rats was 930 units; of five normal rats 1860 units per gram liver). This was so, notwithstanding the fact that a stasis in body weight was produced by the inanition of the normal rats which led to similar final weights in these as in the operated rats. Since the livers are in general slightly heavier in proportion to the body weight, in normal than in hypophysectomized rats, these differences in arginase activity are even greater when expressed as total arginase per 100 gram rat. It must therefore be concluded that hypophysectomy leads to a striking decrease in liver arginase which is independent of any possible effect of the voluntary inanition on the part of the operated rats.

Effect of sex, age and fasting period on liver arginase. Values for the liver arginase activity of about 60 groups of rats of various types are summarized on table 1, for the purpose of comparison. It appears that immature male rats

TABLE 1

Liver arginase of untreated rats

TYPE OF RAT, AGE AT AUTOPSY	NO. OF RATS OR GROUPS*	LENGTH OF FAST	ARGINASE UNITS PER GRAM LIVER	
A: Normal rats				
		hours		
Immature male, 24 days	3 rats	24	1560	*1570*
	3 rats	24	1570	
Immature female, 30 days	5 rats	24	2280	
36 days	5 rats	24	1860	*2010*
44–46 days	5 rats	24	1900	
30 days	3 rats	8	2960	*2840*
	3 rats	8	2720	
Plateaued female, 5–7 months	5 rats	24	2130	*2200*
	1 rat	24	2540	
B: Hypophysectomized rats				
Female, 26–28 days at op., 1–15 weeks p.o.	18 groups	24	1000 ±210†	
Male, 2–3 months at op., 2–4 weeks p.o.	7 groups	24	1020 ±170	
Plat. female, 1 month p.o.	8 rats	24	910	
Female, 26–28 days at op., 9–11 days p.o.	13 groups	8	1320 ±240	
Female, 26–28 days at op., 29 days p.o.	5 rats	8	950	
Female, 26–28 days at op., 2–3 months p.o.	3 rats	8	1150	
C: Adrenalectomized rats‡				
Female, 26–28 days at op., 18 days p.o.	4 rats	24	580	*580*
	2 rats	24	570	

* From 3–8 rats per group.

† Standard deviations for small samples calculated according to: $\sigma = \sqrt{\frac{\Sigma(x^2)}{N-1}}$.

‡ From ref. (3).

(24 days old) show a somewhat lower arginase content than slightly older females (30–46 days old). There does not seem to be any appreciable change with progressing age in the females, the arginase content being similar in the livers of immature and adult plataued rats. These findings are in agreement with the observations of Lightbody who has shown that male rats exceed females

and show increased arginase concentrations only during the period of sexual maturity and activity while females of all age groups showed similar liver arginase contents (8). When the effect of 8 and 24 hours' fasting is compared, the liver arginase concentration is found 25 to 30 percent lower after the longer fast in all types of rats in which relevant data are available (immature females and various kinds of hypophysectomized rats). In view of the loss in liver weight occurring during the 24 hours' fast (about 15 percent), this difference is actually greater when expressed as total liver arginase. Comparison of the enzyme concentration of hypophysectomized animals of different age, sex and postoperative period indicates no differences after 24 hours' fast. After the 8 hours fasting period, two groups which were one to three months postoperative showed lower arginase activities than others which were used one to two weeks after the operation.

Effect of purified pituitary hormones on liver arginase. It has already been reported that adrenocorticotropic hormone (ACTH) increased liver arginase in hypophysectomized rats. This finding has been amply confirmed in rats of various types, with determinations of the arginase after 8 and 24 hours' fasting (table 2, A; 3, A; 5,12–15). The effect was found to parallel other indications of adrenocorticotropic activity, such as increase in adrenal weights (or maintenance when given after hypophysectomy), thymus atrophy and maintenance of carbohydrate stores during fasting. It was found that approximately 1 mgm. of purified ACTH preparations had to be administered daily to produce a significant effect in hypophysectomized rats. In immature normal males, 2 to 5 mgm. hormone were needed. That the effect was independent of the food intake was shown in one experiment in which all animals were fed equally by stomach tube (table 2,A, 5).

Growth hormone was given to a great number of hypophysectomized rats for various time periods (table 4;5). When more than 0.2 mgm. hormone was administered, this principle was found to decrease the liver arginase concentration in all but one experiment. Striking effects could be observed as early as after one day of treatment but doubtful ones after four or five hours. Also in normal plateaued females this hormone markedly decreased the arginase, but was ineffective in immature females at a dose at which also its growth stimulating action was not evident (table 3,B).

Thyrotropic hormone did not affect liver arginase in short term experiments (table 2,B). On the other hand, decreases in liver arginase activity were observed in two experiments in which this hormone was given to hypophysectomized rats for 10 days, starting on the day following operation (table 5,7,8). A low dose of thyroxin appeared to be similarly effective under these conditions (table 5,8a). Lactogenic hormone showed no effect within three or four days (table 2,C). Treatment for 10 to 14 days after hypophysectomy caused variable effects on the arginase concentration (table 2,C; 5,11). A mixture of the two gonadotropins, the follicle stimulating and the interstitial cell stimulating hormone (FSH and ICSH), caused decreases in the arginase activity of hypophysectomized rats in two similar experiments (table 5,9,10).

While the arginase activity has in general been expressed in units per gram liver, it must be kept in mind that various hormones are known to affect liver weights under similar conditions (9). The ACTH in particular, as well as

TABLE 2

Effect of adrenocorticotropic, lactogenic and thyrotropic hormone on liver arginase of hypophysectomized rats, fasted for 24 hours

EXPT. NO.	LENGTH OF TREATMENT	DAILY DOSE	TYPE AND NUMBER OF RATS	LIVER ARGINASE (PER GRAM LIVER)	
				Units	Change
			A: Adrenocorticotropic hormone		
	days	*mgm.*			%
1	2	3.3	3 immat. females, 9 days p.o.	1530	+53
		0.67	3 immat. females, 9 days p.o.	1040	+4
		0.17	3 immat. females, 9 days p.o.	1110	+11
		0.03	3 immat. females, 9 days p.o.	1160	+16
2	3	1.3	3 immat. females, 7 days p.o.	1550	+55
		0.26	3 immat. females, 7 days p.o.	1080	+8
3	3	3.0*	6 immat. females, 3–15 weeks p.o.	1200	+20
4	4	0.25	3 2–3 month old males, 1 day p.o.	880	−12
5	10	1.0*	4 immat. females, 1 day p.o.	1330	+33
6	14	5.0*	5 2–3 month old males, 1 day p.o.	1630	+63
7	14	3.9*	3 2–3 month old males, 1 day p.o.	2700	+170
8	14	3.0*	3 2–3 month old males, 1 day p.o.	2000	+100
9	14	3.0*	4 2–3 month old males, 1 day p.o.	2350	+135
10	14	2.5*	5 2–3 month old males, 1 day p.o.	1800	+80
			B: Thyrotropic hormone		
1	4 hours	1.0	7 plat. females, 5 weeks p.o.	920	−8
2	4 hours	1.0	5 2–3 month old males, 14 days p.o.	1000	0
3	24 hours	1.0	8 2–3 month old males, 14 days p.o.	920	−8
4	24 hours	0.5	7 immat. females, 1 week p.o.	1280	+28
			C: Lactogenic homone		
1	3	3.0	6 immat. females, 3–15 weeks p.o.	1180	+18
2	4	2.5	5 2–3 month old males, 3–15 weeks p.o.	960	−4
3	4	2.5*	4 2–3 month old males, 3–15 weeks p.o.	800	−20
		2.5*	4 2–3 month old males, 3–15 weeks p.o.	1120	+12
4	14	2.0*	5 2–3 month old males, 1 day p.o.	1370	+37
5	14	1.5*	4 2–3 month old males, 1 day p.o.	1800	+80
6	14	1.5*	4 2–3 month old males, 1 day p.o.	1430	+43
7	14	1.25*	5 2–3 month old males, 1 day p.o.	1360	+36

* Half the daily dose given 3 times during the fasting period.

thyrotropic hormone, were found to increase liver weights when given for 10 to 14 days from the day after the operation, while growth hormone is known to delay the growth of the liver when compared with that of the body. Thus both

the increases in liver arginase produced by prolonged administration of ACTH, and the decreases produced by growth hormone (but not those due to the thyroid) would appear even more pronounced were they calculated and expressed in terms of total arginase activity per 100 gram rat.

DISCUSSION. The hypophysis is shown to play a dual rôle in the control of liver arginase activity of rats, just as it is known to do in regard to protein metabolism. The liver arginase increasing action is due to the adrenocorticotropic hormone (ACTH). The evidence that this action is really mediated by the adrenal cortex has been demonstrated elsewhere (3): adrenalectomy caused decreases in liver arginase which were even more marked than those following hypophysectomy, and corticosterone and related steroids were highly effective in increasing liver arginase in normal, hypophysectomized and adrenalectomized rats. This action of the ACTH on liver arginase is in harmony with its established stimulation of protein breakdown and gluconeogenesis.

TABLE 3

Effect of adrenocorticotropic and growth hormone on liver arginase of normal rats

LENGTH OF TREATMENT	DAILY DOSE	TYPE AND NO. OF RATS	LIVER ARGINASE PER GRAM LIVER	
			Units	Change
		A: Adrenocorticotropic hormone		
days	*mgm.*			%
3	10.0	3 21 day old males	1930	+23
	5.0	3 21 day old males	1820	+16
	1.7	3 21 day old males	1500	−4
		B: Growth hormone		
8	3.0	5 plateaued females	1470	−31
	1.0	5 21 day old females	2150	−4

In contradistinction to this, growth hormone was found to decrease liver arginase in hypophysectomized and normal rats. This also is in harmony with the known action of the growth hormone in decreasing the formation and excretion of urea. The fact that thyrotropic hormone and thyroxin may cause decreases in liver arginase activity becomes understandable in view of their ability to produce nitrogen retention (10) and growth in normal rodents (11) and limited weight gains in hypophysectomized rats, at physiological dose levels (9, 12). However, the lack of a rapid action of thyrotropic hormone on liver arginase does not favor this as the mechanism through which thyrotropic hormone lowers blood urea within a few hours (13). The inconsistent effects of lactogenic hormone may possibly be due to variable degrees of ACTH contamination in some of the preparations. The arginase decreasing action produced by a mixture of the purified gonadotropins (FSH and ICSH) is surprising; no effect was found to be produced by physiological or high doses of the female sex hormones (3). It must be kept in mind, however, that the gonadotropins in these experiments

were administered at very high doses when expressed in gonadotropic unitage; while these hormones do not cause general nitrogen retention, it appears possible

TABLE 4

Effect of growth hormone on liver arginase of hypophysectomized rats, fasted for 24 hours preceding autopsy

EXPT. NO.	LENGTH OF TREATMENT	DAILY DOSE	TYPE AND NO. OF RATS	LIVER ARGINASE (PER GRAM LIVER) Units	Change
		mgm.			%
1	4 hours	1.0	6 2–3 month old males, 3 weeks p.o.	1030	+3
	1 day	1.0	6 2–3 month old males, 3 weeks p.o.	780	−22
	1 day	0.2	6 2–3 month old males, 3 weeks p.o.	940	−6
2	1 day	0.5	7 immat. females, 1 week p.o.	840	−16
	1 day	0.5	7 immat. females, 1 week p.o.	960	−4
	1 day	0.1	7 immat. females, 1 week p.o.	1050	+5
3	1 day	3.0	3 immat. females, 1 week p.o.	840	−16
	1 day	0.75	3 immat. females, 1 week p.o.	800	−20
	3 days	0.5*	3 immat. females, 1 week p.o.	830	−17
	3 days	0.5*	3 immat. females, 1 week p.o.	730	−27
4	3 days	1.5*	6 immat. females, 3–15 weeks p.o.	670	−33
5	3 days	2.0*	3 immat. females, 2 weeks p.o.	570	−43
	3 days	0.5*	3 immat. females, 2 weeks p.o.	530	−47
	3 days	0.1*	3 immat. females, 2 weeks p.o.	1000	0
6	3 days	1.5	3 immat. females, 3 weeks p.o.	850	−15
	3 days	0.5	3 immat. females, 3 weeks p.o.	880	−13
	3 days	0.5	3 immat. females, 3 weeks p.o.	900	−10
	3 days	0.2	3 immat. females, 3 weeks p.o.	1060	+6
7	3 days	2.0	3 immat. females, 3 weeks p.o.	1200	+20
8	4 days	0.25*	3 2–3 month old males, 2 weeks p.o.	810	−19
9†	4 days	1.0	3 immat. females, 2 months p.o.	720	−28
	4 days	0.5	3 immat. females, 2 months p.o.	840	−16
10	5 days	0.5*	3 immat. females, 1 week p.o.	760	−24
	7 days	0.5*	3 immat. females, 1 week p.o.	870	−13

* Half the daily dose given 3 times during the fasting period.
† Forty-four hours' fast preceding autopsy.

that their liver arginase decreasing action may be correlated with the tenfold increases in ovarian and uterine weights occurring in these rats. In general it must be concluded that the effects produced by thyrotropic, lactogenic and gona-

dotropic hormones need further investigation and confirmation before definite conclusions may be drawn concerning their mechanism.

In view of the opposed effects on liver arginase activity exerted by various pituitary principles, the removal of this gland might not be expected to produce a marked effect on this enzyme system. Actually, striking decreases in the

TABLE 5

Effect of purified pituitary hormones on liver arginase of hypophysectomized rats, 26–28 days at operation, fasted for 8 hours preceding autopsy

EXPT. NO.	HORMONE PREPARATION	LENGTH OF TREATMENT	NO. OF RATS	DAILY DOSE*	DAYS P.O. AT ONSET	LIVER ARGINASE (PER GRAM LIVER)	
						Units	Change
				mgm.			%
1	Growth	5 hours	3	0.5	7	1110	−16
		32 hours	3	0.5	7	900	−32
		32 hours	3	0.5*	7	870	−34
2	Growth	3 days	3	3.3	7	1070	−19
3	Growth	3 days	3	1.25	7	1050	−20
		3 days	3	0.25	7	830	−37
		3 days	3	0.05	7	1360	+3
4	Growth	4 days	3	1.6	7	810	−38
5	Growth	15 days	9	0.1	14	860	−35
6	Growth	10 days	4	0.5	1	570	−57
7	Thyrotropic	10 days	3	2.0	1	800	−39
8	Thyrotropic†	10 days	3	1.0	1	1030	−22
8a	Thyroxin†	10 days	4	0.0075	1	1010	−23
9	Gonadotropic‡	10 days	3	2.0	1	710	−46
10	Gonadotropic‡	10 days	4	1.0	1	900	−32
11	Lactogenic	10 days	4	1.0	1	790	−40
12	Adrenocorticotropic	10 days	3	2.0	1	1550	+18
13	Adrenocorticotropic	9 days	3	1.0	2	1990	+51
14	Adrenocorticotropic	9 days	3	0.2	2	1420	+8
15	Adrenocorticotropic	15 days	5	3.0*	14	1920	+47

* Injections once daily in all experiments but 1 and 14. In experiment 1 the same dose was given once in the first two groups, 5 and 32 hours preceding autopsy; in the third group this dose was distributed in 4 injections over 32 hours. In experiment 14 the hormone was distributed over 2 daily injections.

† The oxygen consumption was found maintained at normal or slightly supernormal levels in expts. 8 and 8a respectively, the controls being 30 per cent below normal.

‡ A mixture of half FSH and half ICSH.

arginase were regularly observed following hypophysectomy; through paired feeding experiments, this response was shown not to be due to the low food consumption of hypophysectomized rats. Light was thrown on this question through a comparison of the relative dose levels of the two main "opponents," ACTH and growth hormone, required for the production of changes in liver arginase activity. Of the ACTH about 1 mgm. has to be administered daily to hypophysectomized rats to cause this or any other functional effect, such as

maintenance of carbohydrate stores during fasting, thymus atrophy, etc.[3] This same dose also prevents adrenal atrophy when given from the day following the operation. It can therefore be concluded that the action of ACTH in increasing liver arginase is produced by a physiological dose, i.e., by an amount of hormone equivalent to that secreted by the intact hypophysis. On the other hand, growth hormone is effective in decreasing liver arginase only at doses of 0.1 to 0.5 mgm. daily, although these preparations at one-tenth of this dose level produce marked weight gains in such hypophysectomized rats. It thus appears probable that the normally functioning pituitary gland does not secrete enough growth hormone to balance the action of ACTH in regard to arginase concentration. It then becomes understandable why the overall effect of removing both hormones, i.e., hypophysectomy, causes a fall in arginase concentration. On the other hand, the finding that adrenalectomy leads to lower liver arginases than occur after hypophysectomy (3) can be regarded as further evidence for the existence of a pituitary principle with an action opposed to that of ACTH. The question poses itself whether the pituitary controls nitrogen metabolism through its action on arginase concentration or whether the changes in arginase activity represent secondary adjustments to an altered metabolism. In regard to the rôle of the adrenal, and thus of ACTH, evidence will be presented elsewhere (3) favoring the view that arginase concentration may actually represent one of the points of attack through which this gland accelerates protein breakdown. On the other hand, the finding that growth hormone stimulates growth at levels which are considerably lower than those necessary to produce appreciable changes in the arginase concentration does not favor a similar interpretation for the action of this hormone. It appears here more likely that the arginase is decreased secondarily in response to a lessened need for this enzyme in animals with a positive nitrogen balance.

SUMMARY

1. Hypophysectomy was shown to lead to a marked decrease in liver arginase activity.

2. This process could be reversed by the administration of adrenocorticotropic hormone. This pituitary hormone also increased the arginase activity of the livers of normal rats.

3. In contradistinction to this, growth hormone was found to decrease the arginase activity in hypophysectomized and normal rats.

4. The possible physiological significance of these newly discovered functions of the anterior hypophysis was discussed.

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STUDIES ON HEMOCONCENTRATION AND SHOCK FOLLOWING SEVERE HEMORRHAGE[1]

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Whether severe hemorrhage can lead to true shock is still controversial, as the recent literature reveals. If the reduction in blood volume which follows severe hemorrhage fails to produce shock, the theory which attributes the development of traumatic shock to oligemia, resulting from local fluid loss at the site of injury, would be weakened considerably. The work presented here may contribute to the clarification of this problem by demonstrating that shock, with its characteristic manifestations as set forth by Moon, can follow hemorrhage under certain experimental conditions.

Some investigators (Moon; Coonse *et al.*; Mahaffey)[2] have reported that hemodilution invariably follows hemorrhage and have used this observation as the basis for a differentiation between the effects of hemorrhage and shock, emphasizing that, after hemorrhage, hemoconcentration may occur only terminally. More recently, Moon (1) again has stressed that, despite similarities, hemorrhage and shock should be differentiated, clinically and experimentally, lest erroneous conclusions be drawn. Price (2) concurred that in normal anesthetized dogs severe hemorrhage always produces hemodilution and that the outstanding physiologic and pathologic effects of acute hemorrhage are not found in shock produced by other means.

Others (Blalock; Freeman; Davis)[2] have contended that after severe hemorrhage all of the postulated signs of shock, including hemoconcentration, may develop. Blalock (3) and Harkins (4), in their recent reviews, conclude that the differentiation of hemorrhagic from other types of shock is unsound, and that the sequelae of post-hemorrhagic oligemia may include hemoconcentration, if sufficient time is allowed to elapse before death or therapy.

In the light of Starling's theory of fluid exchange in the capillary bed, some hemodilution must necessarily occur after hemorrhage, due to the shift of body water into intravascular circulation as the capillary hydrostatic pressure falls. In well hydrated animals, the early hemodilution following hemorrhage is so great that it can either compensate for extensive blood loss or else (partially or totally) mask subsequent hemoconcentration. It was felt that if the effects of such hemodilution were reduced by deprivation of water, hemoconcentration might be demonstrated to occur after graded bleeding, a procedure by which the fluid reserves of the body are known to be depleted severely (5).

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[2] All references without index numbers can be found in reviews 3 and 4.

METHODS. Twenty-six healthy, adult, unanesthetized mongrel dogs, weighing from 5.7 to 22 kgm., were used. The animals were starved for 30 hours. Eleven dogs were given water ad libitum. The 15 "dehydrated" dogs were deprived of water for 18 to 30 hours and, in the cooler weather, were exercised for 30 minutes the day before the experiment.

Blood pressures were recorded with the glass capsule manometer (6), which gives a fairly good indication of pulse pressure. Heparin was used as the anti-coagulant. The following determinations were performed in duplicate: circulating time by the sodium cyanide method (7); *thiocyanate dilution* by the method of Crandall and Anderson (8); plasma volume by the "direct" dye method (9), using the spectrophotelometer, calibrated syringes and washing out the syringes with blood after dye injections; plasma protein determinations by the micro-Kjehldal method of Ma (10); non-protein nitrogen by direct Nesslerization (11); arterial CO_2 by the manometric method of Van Slyke and Neill; hematocrits with Wintrobe tubes, centrifuged for one hour at 2,500 R.P.M.; hemoglobins with a photoelectric hemoglobinomcter; and red cell counts in the usual manner. Blood samples were taken after draining the stagnant blood from the cannulas.

The experimental procedure was as follows: Under local (procaine) anesthesia, the right femoral and right carotid arteries were cannulated and the left femoral and the right external jugular veins were exposed. A control (arterial) blood sample of 15 to 20 cc. was drawn, and sodium thiocyanate solution was injected intravenously. Then, carotid arterial blood pressure and circulating time were recorded. Forty-four minutes after the injection of the thiocyanate, the blue dye was injected. Sixteen minutes later, the first dye sample was drawn from the femoral artery, and, at 4 or 5 minute intervals, 3 or more successive samples were drawn. Hematocrit determinations were made on alternate dye samples. Immediately after the last sample was drawn, the animal was bled a total of 20 to 30 per cent of its estimated blood volume in a 10 minute period. Blood volumes were calculated as $\frac{1}{13}$ of body weight. Carotid blood pressures were recorded during the bleeding periods. Then the arteries were clamped and the animal was placed on the floor, restrained only by a leash. After about thirty minutes the compensation to the hemorrhage was assumed to be complete (12), and the animal was bled an additional 10 to 15 per cent of its estimated blood volume in a 10 minute period. In this and in all successive bleedings if the mean arterial pressure fell below 45 to 50 mm. of Hg, the bleeding was stopped and the animal removed from the board; thirty minutes later, a third bleeding was performed if the animal's condition permitted it.

Circulatory collapse from hemorrhage or trauma was generally characterized by a persistent fall in the blood pressure to less than 70 mm. Hg, by a progressive increase in the circulating time to 20 seconds or more, and by a decrease in the arterial carbon dioxide content to less than 26 volumes per cent. In this investigation, an animal was considered to be in shock only when at least two of these three criteria were present. Generally, the changes in all three were parallel but, not infrequently, one would give more or less normal values when the other two gave a more accurate picture of the animal's critical condition.

After an average period of 35 minutes following the final hemorrhage, the animals were in shock; a 15 cc. sample of blood was drawn for the various determinations and the dye was injected for the second plasma volume determination. All of the animals died some time after the final hemorrhage unless serum or plasma infusions were administered.

RESULTS. The experiments are divided into two main groups, "non-dehydrated" and "dehydrated" animals.

TABLE 1

Average values for all experiments

	NON-DEHYDRATED ANIMALS		DEHYDRATED ANIMALS	
	Hemodiluting	Hemoconcentrating	Hemodiluting	Hemoconcentrating
No. of dogs	9	2	7	8
Wt. (kgm.)	14.7	8.2	10.7	10.2
"Extra-cellular" fluid (cc./kgm.)	256	253	227	227
Plasma volume (cc./kgm.)	36.0	42.0	36.4	38.5
Circulating blood volume (cc./kgm.)	73.6	79.4	74.3	75.3
Total hemorr.				
Cc./kgm	36.0	35.7	32.2	32.0
% of control blood volume	49.0	44.9	43.3	42.5

	TIME OF DETERMINATION							
	Control	Post-hemorr.	Control	Post-hemorr.	Control	Post-hemorr.	Control	Post-hemorr.
Hematocrit	51.6	43.3	50.2	54.6	50.7	45.2	48.9	53.5
Hemoglobin (grams %)	18.1	14.7	17.5	19.3	17.5	15.4	16.2	17.8
Erythrocyte count (10^6/mm.^{3})	7.8	6.4	6.9	7.6	7.1	6.0	6.6	7.5
Mean blood pressure (mm. Hg)	148	58	121	47	126	51	140	48
Circulating time (seconds)	10.4	38.5	14.5	32.0	9.0	42.3	9.6	39.5
Arterial CO_2 (vol. %)	46.4	15.7	39.0	5.1	44.2	17.9	46.6	16.8
NPN (mgm. %)	32	44	47	62	41	59	40	60
Plasma protein (grams %)	5.8	4.7	6.3	5.2	6.1	5.0	6.7	5.9

Hemoconcentration occurred in 2 of the 11 "non-dehydrated" and in 8 of the 15 "dehydrated" animals, the average increase in hematocrit being 4.4 and 4.6, respectively. The average decrease in hematocrit for the 7 dehydrated hemodiluting animals was 5.5, whereas in the 9 non-dehydrated hemodiluting animals it was 8.3. The changes in hemoglobin and red cell counts were in the same direction.

The total blood loss which led to shock differed significantly in the various groups. By total blood loss is meant all measured blood lost in hemorrhages, samples, washing out cannulas, etc. The non-dehydrated hemodiluting dogs withstood an average withdrawal of nearly 50 per cent of their average control blood volume, whereas both groups of dehydrated dogs and the non-dehydrated

hemoconcentrating dogs tolerated an average loss of only 43 per cent of their average initial blood volume.

The changes in total plasma protein concentration revealed that in the dehydrated hemoconcentrating group the decrease in the concentration of total protein, after hemorrhage, was significantly less than in the other groups.

An increase in non-protein nitrogen concentration was observed in every animal except in one of the hemodiluting, non-dehydrated group. The grestest increase (50 per cent) was found in the group of dehydrated hemoconcentrating animals.

Average mean blood pressures, 30 minutes after the last hemorrhage, were at shock levels in all groups. Significantly, the blood pressures were highest in the non-dehydrated, hemodiluting animals, although this group had been subjected to the severest hemorrhage (nearly 50 per cent of the average initial blood volume). Pulse pressures were greatly reduced, indicating decreased cardiac output and depleted circulating volume. The importance of low pulse pressures in the diagnosis of shock has recently been given new emphasis (13).

Arterial blood CO_2 content was decreased to very low levels in the animals of all groups.

Circulating time was markedly increased in every animal.

The general condition of the animals when shock was present appeared to be the same in all four groups. It seemed impossible to differentiate dehydrated from non-dehydrated or hemodiluting from hemoconcentrating dogs. All the animals in shock were listless, not responsive to stimuli, and unable to walk. Severe diarrhea occurred in a number of animals of each group and, often, was bloody. In animals in this condition, withdrawing a 10–15 cc. blood sample frequently induced a terminal circulatory collapse within a few minutes. In several such cases, infusion of 5 per cent dextrose in lactate Ringer's solution, in amounts greater than the total volume of blood lost, was attempted but failed to restore the circulation and death soon occurred.

"Extra-cellular" fluid volumes, as measured by thiocyanate dilution, varied too much in the individual dog to permit correlations to be made. It is interesting to note that, despite great individual variations in both groups, the average value for the dehydrated animals was 227 cc./kgm., whereas the average value for the non-dehydrated animals was 255 cc./kgm., both values at the lower range of the normal limits of 230 to 425 cc. per kgm. (14).

Plasma volume values before hemorrhage in all of the animals fell within usual normal ranges (14). We have confirmed the findings of others (15, 16) that plasma volumes, measured by the blue dye, not only give a good index of the clinical condition of the animal, but also correlate well with the other determinations (v.s.) and thus seem to be a reliable measure of the intravascular plasma volume in shock animals. However, because of the uneven distribution of red cells in the circulation and of the unknown amounts of non-circulating red cells, calculated total red cell volumes and total blood volumes are very inaccurate (2, 17) and, consequently, are quantitatively not significant.

In table 2 a comparison of the average percent change in the plasma volume after hemorrhage with the average percent of the control blood volume which

was withdrawn during the period between the first and the second plasma volume determinations indicates that the animals which hemoconcentrated apparently lost plasma fluid and plasma protein in addition to that removed in the hemorrhage, whereas the animals which hemodiluted apparently drew protein-poor fluid into the circulation, during and after the hemorrhage, and had retained it at the time the second plasma volume was determined. The apparent change in circulating red cell volume, and, consequently, the change in total blood volume are less significant for the reasons mentioned above. The validity and interpretation of the apparent loss of additional red cells by the hemodiluting animals and the relative gain in red cells in the hemoconcentrating animals are not established, as yet.

TABLE 2

Average volumes for experiments in which post-hemorrhage plasma volumes were determined

	NON-DEHYDRATED		DEHYDRATED	
	Hemodiluting	Hemoconcentrating	Hemodiluting	Hemoconcentrating
No. of dogs	5	1	5	5
Hemorr. between control and post-hemorrhage plasma volume determinations				
Cc./kgm.	29.3	36.0	28.8	29.3
% of control blood volume	39.8.	39.8	36.8	38.2

	CONTROL	POST HEM.	% DECREASE	CONTROL	POST HEM.	% DECREASE	CONTROL	POST HEM.	% DECREASE	CONTROL	POST HEM.	% DECREASE
Plasma volume (cc./kgm.)	36.0	26.2	27.2	45.1	24.4	46	37.5	26.4	29.5	41.0	23.8	42.6
Circulating blood volume (cc./kgm.)	74.0	46.1	37.6	90.2	55.7	39.3	78.3	51.7	34.0	76.8	47.0	38.8
Total circulating protein (grams/kgm.)	1.9	1.13	40.5	2.57	1.0	61.1	2.43	1.54	37.0	2.59	1.21	54

Pathological findings were variable, as others (13) have reported. Only 11 animals which did not receive infusions will be considered. The gross findings were not consistent in all cases of each group. Generally, the gut was moist, flabby and atonic, and the lumen often contained gross blood. The intestinal mucosa often was engorged and hemorrhagic throughout. The peritoneal and pleural cavities rarely contained increased amounts of free fluid, and, in only one case, was this blood-stained. The lungs, in some dogs, were engorged and moist but, generally, they presented a fairly normal gross appearance. Nothing of significance was found on gross inspection of the other viscera. The outstanding microscopic findings were focal congestion and engorgement of the lungs, marked engorgement and central necrosis in the liver, marked engorgement of the zona fasciculata of the adrenals, and marked engorgement of the intestine and kidney.

DISCUSSION. Hemoconcentration, as measured by hematocrit, red cell count, hemoglobin, etc., has been advanced as a *sine qua non* of shock. Starting from the premise that hemorrhage is followed by hemodilution, Moon (1) has warned investigators not to apply incorrectly to "true" shock conclusions drawn from animals subjected to hemorrhage. Our results differ markedly from his in certain important respects, for by uncomplicated graded hemorrhage we have produced all of the symptoms and most of the important signs which he contends appear only in "true" shock. Moreover, these findings were observed in a number of animals long before death and in others which survived after serum or plasma infusions, and, therefore, cannot be dismissed as pre-terminal phenomena. That hemoconcentration appeared in more animals of the dehydrated group does not affect the conclusion that the oligemia produced by severe hemorrhage, alone, may be sufficient to produce shock.

Adolph (12) has demonstrated that it is unreliable to use the changes in the peripheral blood red cell concentration as estimations of loss or gain of fluid from the circulation, since stored red cells can be shifted rapidly into or out of active circulation. Thus, an apparent hemoconcentration may mean not a loss of plasma but a gain of cells by the circulating blood. However, with T-1824, plasma volumes can be determined fairly accurately and, by a comparison of the observed plasma volume after hemorrhage with the expected plasma volume, calculated by subtracting from the initial plasma volume the amount of plasma removed in hemorrhage, quantitative estimations of fluid shifts can be derived. In 37 anesthetized dogs which hemodiluted after prolonged bleeding, Price (2) reported a significant average gain of fluid. Similar calculations led us to the conclusion that our dogs which hemoconcentrated actually lost fluid from the blood stream in addition to that lost by hemorrhage, whereas the hemodiluting animals tended to retain fluid gained after the hemorrhage. From this it is clear, therefore, that the hemoconcentration which we observed in a number of dogs was due to the loss of additional fluid from the circulation after hemorrhage.

From the total circulating protein before and after hemorrhage (table 2) and the amount of protein removed in bleeding, it can be calculated that the animals which lost fluid actually lost protein from the circulation, too. If allowance is made for this protein loss, the decrease in plasma protein concentration after hemorrhage in the hemoconcentrating animals indicates that initially, like the hemodiluting animals, these animals must have drawn a relatively protein-poor fluid into the circulation. Thus the total fluid ultimately lost, after the animals began to hemoconcentrate, actually must have been greater than the amounts calculated.

These changes in plasma protein concentration also indicate that the dehydrated hemoconcentrating animals could hemodilute less than all the others. Calvin (18) has shown that, in response to moderate hemorrhage, dehydrated dogs can hemodilute less than normal ones. This possibly explains the inability of our dehydrated animals to tolerate as much bleeding as the hydrated animals before going into shock. This, also, may have decreased the masking effect of the earlier hemodilution upon the later outpouring of fluid from the circulation as shock develops and progresses.

One important question remains: why did 2 of the 11 non-dehydrated dogs display hemoconcentration and why did only 8 of the 15 dehydrated dogs display hemoconcentration? This may be part of the broader problem of why some investigators have been able to observe hemoconcentration following hemorrhage and others not. Blalock (3) and Harkins (4) have stressed the possibility that this discrepancy may depend on the time factor, stating that sufficient time must elaspse after hemorrhage before hemoconcentration can occur. But whether hemoconcentration will occur after hemorrhage may depend as much, or even more, on the state of hydration of the animal and on the physiological and pathological variables which affect hydration. A supposedly "normal," non-dehydrated animal actually may be dehydrated and in poorer condition than a healthy young animal that has been dehydrated experimentally for 30 hours. Until more accurate means of determining intra-cellular and extra-cellular water are developed, the evaluation of the factor of hydration in an individual case will be difficult. Our group averages for "extra-cellular" fluids (i.e., thiocyanate dilution) point in that direction, however.

Under the stress of modern warfare, dehydration of combatants may occur and severe hemorrhage may produce shock with hemoconcentration. It would seem to be unwise, therefore, to differentiate hemorrhagic shock from shock produced by other causes. Further, our studies indicate that the appearance of hemoconcentration following hemorrhage is a *signum male ominis*. The clinician who believes that only hemodilution can follow hemorrhage, in the absence of hemodilution, may have a false sense of security in the belief that the patient has not lost much blood. The opposite may actually be the case. In most instances, fortunately, the prevailing clinical picture will give adequate warning of the patient's critical condition.

SUMMARY

1. Typical shock has been produced by graded hemorrhage in 11 non-dehydrated, and in 15 dehydrated, unanesthetized, normal dogs.

2. In 2 of the non-dehydrated (18 per cent) and in 8 of the dehydrated animals (53 per cent), hemoconcentration occurred. Plasma volume and plasma protein determinations, before and after hemorrhage, revealed that the animals which hemoconcentrated, actually lost additional plasma fluid and protein as shock developed.

3. Pathologic changes consisting of gastro-intestinal engorgement and hemorrhage, pulmonary congestion and engorgement, and occasional changes in other viscera were observed in a number of animals.

4. The hemodiluting, non-dehydrated animals tolerated an average total blood loss of 49 per cent as compared to an average total blood loss of 43 per cent tolerated by the other animals.

5. The changes in plasma protein concentration after hemorrhage indicated that the hemoconcentrating dehydrated animals hemodiluted during and after hemorrhage to a lesser degree than the other 3 groups. This relative inability to hemodilute could explain their inability to tolerate as much bleeding

before going into shock and could lessen the masking by earlier hemodilution of he subsequent hemoconcentration as shock develops.

6. It is suggested that the conflicting reports as to the occurrence of hemoconcentration after hemorrhage may be related to the state of hydration of the animals studied.

7. It is concluded that there are no definite grounds for differentiating between hemorrhagic shock and shock from other causes.

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THE EFFECT OF NEMBUTAL-ETHER ANESTHESIA UPON BLOOD CONCENTRATION

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The correct interpretation of experimental data obtained from anesthetized animals requires an understanding of the physiological abnormalities induced by the narcotic agents employed. Important dislocation of fluid balances is known to occur under anesthesia. Thus, etherized dogs show hemoconcentration (1, 2, and others) associated with a significant fall in circulating plasma volume (3). Several barbiturates on the other hand produce hemodilution in this animal (4). The cat also shows increased plasma volume under nembutal anesthesia (5), but it differs from the dog in that hemoconcentration does not occur with ether (6). Since it seemed possible that ether might reconcentrate the blood of cats previously diluted by nembutal, the effect of combining the two drugs has been studied.

Methods. Dogs and cats were used in this investigation. Nembutal (30 to 36 mgm. per kgm.) was injected intravenously or intraperitoneally. Ether was administered by means of a positive pressure respirator, thus assuring adequate oxygenation of the blood. Blood ether determinations (7) made upon several animals showed that anesthetic concentrations of ether were present. Blood was drawn without stasis from the saphenous veins of cats and from the jugular veins of dogs. After the heparinized blood had been centrifuged in Wintrobe hematocrit tubes, the supernatant fluid was used for protein determinations. The protein percentage obtained by the refractometer method was checked in many instances by the falling drop technique. Plasma volume measurements were made with the blue dye, T-1824, according to Gregersen et al. (8). All operations were carried out with strict aseptic precautions.

Results. In 24 experiments on 14 cats anesthetic doses of nembutal produced significant decreases in the hematocrit values (average 26.5 per cent) and in the plasma protein concentrations (average 16 per cent). These changes, which suggest that blood dilution has occurred, are in agreement with the report that nembutal increases the plasma volume in the cat (5).

Since it is difficult to obtain blood from unanesthetized cats without exciting the animals, it seemed necessary to rule out the possibility of emotional hemoconcentration preceding the nembutal dilution. This factor was excluded in three experiments by drawing control samples with the animal under ether anesthesia. Under these conditions the usual nembutal dilution occurred. Conley's (6) experiments as well as the following observations demonstrate that the ether did not affect the blood concentration.

The effect of the nembutal anesthesia followed by the administration of ether was studied in eight cats (10 exps.). After control blood had been taken,

the animals were anesthetized with intravenous injections of nembutal. Three blood samples were obtained at 10-minute intervals, and then ether was administered. Nembutal consistently caused a decrease in the hematocrit readings and in the plasma protein concentrations. These values were essentially unchanged by 40 to 60 minutes of ether inhalation (see fig. 1). Control studies showed that the changes in intrathoracic pressure induced by the respirator were without effect upon the results.

The above experiments were compared with a series in which dogs were used. The intravenous injection of nembutal into 3 normal dogs resulted in a fall in the hematocrit values (average 22.9 per cent) and a decrease in the plasma protein concentrations (average 9.3 per cent). In 11 experiments on 5 normal dogs nembutal anesthesia was followed by the administration of ether. Under these conditions the hemodilution (as measured by hematocrit readings and plasma

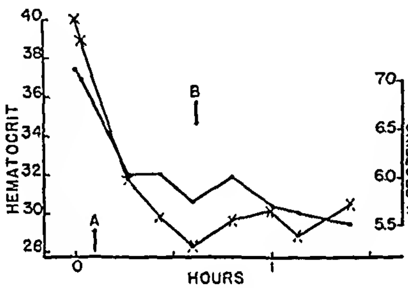

Fig. 1

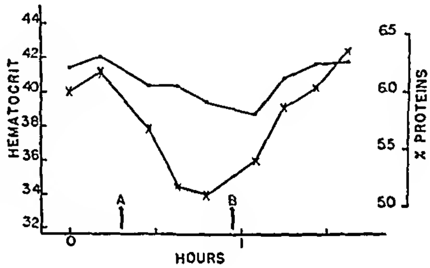

Fig. 2

Fig. 1. The effect of ether inhalation, *B*, on the blood concentration (● = plasma proteins, × = hematocrit) of a normal cat previously anesthetized by means of nembutal, *A*.

Fig. 2. The effect of ether inhalation, *B*, on the blood concentration (● = plasma proteins, × = hematocrit) of a normal dog previously anesthetized by means of nembutal, *A*.

protein concentrations) produced by nembutal injection was succeeded by a marked ether hemoconcentration. Figure 2 shows a typical experiment.

In attempting to explain this discrepancy in the effect of ether one must consider the differences in circulatory response to this anesthetic exhibited by the two species. Thus, it is reported that ether produces vasoconstriction in the hind limbs of the cat (9), whereas in the dog it causes an increased blood flow in the femoral arteries (10). It is possible that femoral vasodilatation may so alter the hemodynamic relations that fluid loss from the circulation is favored. If this is the explanation for ether hemoconcentration in the dog, the same phenomenon should occur in cats which have been deprived of the sympathetic innervation of the hind limbs. However, in 3 cats from which the abdominal sympathetic chains had been removed, ether anesthesia produced no significant changes in hematocrit or plasma protein concentrations. Moreover, ether still failed to produce hemoconcentration in one of these animals after bilateral adrenalectomy.

A further investigation into the difference in the response to ether anesthesia exhibited by cats and dogs was carried out on a number of splenectomized ani-

mals. Seven splenectomized cats responded to nembutal with an average fall of 11.8 per cent in plasma proteins and of 11.2 per cent in hematocrit. In three animals the circulating plasma volume, as determined by the blue dye, T-1824, showed an average increase of 10.1 per cent. The administration of ether 30 minutes after nembutal injection (5 exps.) produced no discernible effect upon the hematocrit, the plasma proteins, or the plasma volume.

In 8 experiments on 7 splenectomized dogs the intravenous injection of nembutal caused an average fall of 4 per cent in plasma proteins and of 3.3 per cent in the hematocrit values. Under these conditions the average increase in the circulating plasma volume was 4 per cent. When ether was administered during the nembutal anesthesia, the plasma protein concentration and the circulating plasma volume returned to the control values, but the hematocrit readings exceeded the control values by about 4.5 per cent.

Discussion. These experiments demonstrate that the injection of nembutal produces hemodilution in normal dogs and cats. In both species the hematocrit values fall to a greater extent than do the plasma protein concentrations. The decrease in protein concentration can be accounted for by augmentation of plasma volume by entering fluid (5), for there is no reason to expect true loss of protein under the conditions of these experiments. The excess fall in hematocrit not accounted for by increased plasma volume indicates egress of cells from the circulating stream, decrease in volume of the erythrocytes, or both. The fact that splenectomy abolishes the difference between hematocrit and protein dilution suggests that segregation of cells in the spleen, dilated by nembutal (see 4), is the important factor. In addition to this activity as erythrocyte reservoir the spleen seems also to be concerned in the movement of the fluid, for splenectomy reduces the amount of protein dilution occurring under nembutal anesthesia. This reduction occurs in both dogs and cats, but is much more marked in the former.

In the dog these changes in blood concentration induced by nembutal are reversed in direction by subsequent etherization: The plasma protein values rise, but the hematocrit is increased even more. The changes are comparable with those resulting from ether anesthesia alone (3). In this case, then, fluid leaves the circulation, while erythrocytes enter it. Splenectomized dogs exhibit the same response to ether, including the excess rise in hematocrit over plasma protein. This would seem to indicate that in the splenectomized dog previously anesthetized with nembutal ether empties some extrasplenic cell reservoir, or else the drug causes a redistribution of erythrocytes in such a manner as to produce an apparent rise in the venous hematocrit. Possible changes in the volume of the individual red cells undetected by hematocrit measurements must once again be mentioned.

The administration of ether to normal and splenectomized cats already under nembutal anesthesia produces no change in plasma volume, plasma protein concentration, or hematocrit values. Ether, therefore, fails to raise the hematocrit in the cat not only normally (5) but also when the animal's spleen has already been dilated (4) by nembutal anesthesia. The failure of the previously un-

anesthetized cat to respond to ether by increase in the hematocrit, in the manner exhibited by the dog, is therefore not attributable to pre-existing maximal contraction of the spleen. The spleens of the two animals apparently react in a different manner to ether. A species difference also exists in the effect of ether on the plasma volume. In dogs, with or without preceding nembutal anesthesia, splenectomized or normal, ether causes hemoconcentration, whereas it fails to change the plasma volume of cats even when the volume has been increased by nembutal. Either the mechanisms which control fluid transfer are not the same in the dog and the cat, or else the action of ether differs in the two species.

SUMMARY

1. In both dogs and cats nembutal anesthesia produces a marked decrease in hematocrit (cats 26.5 per cent, dogs 22.9 per cent) and plasma protein concentration (cats 16 per cent, dogs 9.3 per cent).

2. After splenectomy nembutal anesthesia no longer produces a greater decrease in the hematocrit value than in the plasma protein concentration. The magnitude of both changes is decreased in the splenectomized animal.

3. In dogs anesthetized with nembutal ether raises the previously lowered hematocrit and protein readings above control levels. A similar response occurs in splenectomized dogs.

4. Neither normal nor splenectomized cats anesthetized with nembutal show any evidence of changes in blood concentration in response to ether.

5. The same lack of reaction occurs in cats from which the abdominal sympathetic chains or the chains and both adrenals have been removed.

I should like to thank Dr. Walter S. Root for his constant advice and assistance in these experiments.

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THE MECHANISM OF BILE FLOW INHIBITION UPON DISTENTION OF THE COLON OR STIMULATION OF ITS NERVE SUPPLY

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Goldman and Ivy (1) found that distention of the colon in the dog and monkey caused inhibition of hepatic bile flow. A similar inhibition occurred upon electrical stimulation of the colonic, inferior mesenteric, superior mesenteric, and pelvic nerves. No inhibition was observed by these methods after sectioning of the hepatic nerves. Kuntz and Van Buskirk (2) found that bilateral sectioning of the vagi and splanchnic nerves had no effect on the response which, however, was abolished by application of nicotine to the celiac ganglion. They suggested that the celiac ganglion was a true reflex center. However, in their experiments the lumbar sympathetics were left intact.

The present paper confirms the occurrence of inhibition of bile flow upon colonic stimulation, after section of the vagi and splanchnic nerves, and presents evidence regarding whether the celiac ganglion is a reflex center.

Methods. We followed the procedure of Goldman and Ivy. The inhibition of hepatic bile flow was studied in 31 dogs under sodium pentothal anesthesia. Ether anesthesia, with morphine and atropine as preanesthetic medication. was also tried, but had no advantage over sodium pentobarbital. The common bile duct was cannulated near the duodenum, and the cystic duct was tied. To distend the colon with water (at 40°C.), the sigmoid colon was tied and a cannula was placed into the cecum or the appendix. Extreme care was exercised at all times to prevent kinking or other obstruction of the bile duct system. During distention the colon was kept outside the abdominal cavity with an intraluminal pressure of 100 to 120 cm. of water for 5 minutes. By means of an electric drop-counter a record of bile flow was kept on a kymographic tracing, which also recorded the blood pressure. For electrical excitation of the colonic nerve or the inferior mesenteric ganglion, a shielded electrode was applied. Stimulations lasted 5 minutes.

After inhibition of bile flow was established, we sectioned the vagi and splanchnics and removed the lumbar chains of sympathetic ganglia. One or more of these lesions were performed in any one dog. Since extensive autonomic nerve lesions at any one operation cause a severe drop in blood pressure, we sectioned the right splanchnics and removed the right lumbar chain under aseptic conditions in a series of animals; such dogs were then studied under anesthesia as described above, from two to three weeks postoperatively.

Results. A. *Dogs Without Any Nervous Lesions. Colon distention.* Out of 12 dogs studied, two gave no bile flow, even after intravenous injection of 3 cc.

of 20 per cent solution of sodium dehydrocholate. Of the 10 "normal" dogs which had a bile flow, colon distention inhibited this flow in 7, no effect being obtained in the remainder. The degree of inhibition of bile flow varied from a slight slowing of flow to a cessation of flow for several minutes.

Electrical excitation of nerves. Out of 10 dogs studied (not the same animals as reported above), one dog had no bile flow at all. Of the other 9 dogs, two showed inhibition of bile flow; the other 7 showed no effect. Because of the irregularity of the response, this method of approach to the study of the reflex activity of the celiac ganglion was discontinued.

B. *Dogs with Nerve Lesions. "Acute" nerve lesions.* Bilateral vagotomy in the neck in two dogs did not prevent inhibition of bile flow upon colon distention. Section of the right splanchnics and removal of the right lumbar sympathetic chain in 5 dogs caused such a drop in blood pressure that responses to distention were unsatisfactory and uncertain. Bilateral section of the splanchnics and bilateral removal of the lumbar chains at a single operation caused death from shock in 4 dogs. Because complete severance of the nerves connecting the celiac ganglion with the medulla and spinal cord caused "shock" so frequently, this approach had to be discontinued.

Chronic nerve lesions. In 8 dogs the right splanchnics and right lumbar chains were removed (checked by autopsy) aseptically 3 weeks before the acute experiment. Of 8 dogs, 4 showed inhibition of bile flow upon colon distention. The 4 other dogs had a normal bile flow which could not be inhibited by colon distention.

Since vagotomy, as observed above, does not abolish the inhibitory response, the response observed in these experiments may have occurred *a*, via fibers in the left lumbar chain and splanchnic fibers; *b*, via a true ganglion reflex; *c*, via a ganglionic pseudo-reflex, or *d*, via the blood stream. To rule out possibility *a*, the left lumbar chain and splanchnic fibers were excised.

Two of the preceding dogs, which showed inhibition on colon distention, still showed inhibition of bile flow after the left splanchnics and left lumbar chains were sectioned and excised completely, as confirmed by autopsy. In addition, the vagi were cut in one of these animals.

General observations. Among the conditions which were necessary for a successful inhibitory response, the manner of cannulating the colon was important. Preliminary experiments indicated that the cecal region, including the appendix, most readily gives rise to inhibition of hepatic bile flow. When the colon was distended distal to the cecum, we observed no inhibition of bile flow. Another important factor was the trauma inflicted on the wall of the colon by numerous distentions. If several distentions were necessary to establish the occurrence of inhibition "normally," it was believed that failure to obtain inhibition of bile flow after nerve lesions and further distentions in such an animal could not with certainty be ascribed to the section of the nerves.

The observation was repeatedly made that inhibition of bile flow was even more marked *following* the end of stimulation (distention or nerve excitation) than during the period of stimulation.

DISCUSSION. The results show that distention of the proximal colon of the dog causes inhibition of hepatic bile flow, even after bilateral vagotomy and splanchnic section and bilateral excision of the lumbar sympathetic chains. (Removal of the right lumbar chain with section of the right splanchnic nerves from 2 to 3 weeks previous to the distention of the colon appears to abolish reflex inhibition of hepatic bile flow in some, but does not do so in all dogs.) It follows that the inhibition must be due to either *a*, a true ganglionic reflex; *b*, a ganglionic pseudo-reflex (3); or *c*, a hormone carried by the blood stream. Since it was clearly shown that section of the hepatic nerves abolishes the reflex, a hormone mechanism is ruled out (1). The abolition of the reflex by treatment of the celiac ganglion (2) with nicotine shows that the ganglion is the reflex center for the response, but it does not show whether the reflex is of the nature of a true reflex or a pseudo-reflex. To determine which of the two possible types of reflex activity is concerned, it would be necessary to distend the colon in animals in which the vagi, splanchnic and lumbar sympathetic nerves have been sectioned for a sufficient period of time to permit degeneration of branched visceral sensory axones. It would seem odd, however, for a sensory visceral nerve with a cell body located in the dorsal root ganglion to send one axone branch to the celiac ganglion and another to the proximal colon. Such a concept is more plausible morphologically in the case of Langley and Anderson's (3) observation on the pseudo-reflex of the urinary bladder in which the center is located in the inferior mesenteric ganglion.

CONCLUSIONS

1. Distention of the proximal colon in the dog inhibits hepatic bile flow in about 70 per cent of anesthetized dogs. Inhibition from stimulation of the colonic nerves or the inferior mesenteric ganglion was not so frequently observed in these as in previous experiments.

2. The reflex concerned may be excited after decentralization of the celiac ganglion, by section of the vagi, splanchnic nerves, and excision of the lumbar sympathetics.

3. The evidence strongly indicates that the celiac ganglion is either a true or a pseudo-reflex center for the temporary inhibition of hepatic bile flow which occurs on distention of the proximal colon. The nerve fibers concerned are not located in the vagi, but in either the thoraco-lumbar sympathetics or the prevertebral autonomic system.

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EXCRETION OF THE URINARY ANTIDIURETIC[1] PRINCIPLE IN RENAL HYPERTENSIVE DOGS

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Studies of the possible rôle of the posterior pituitary in the pathogenesis of experimental renal (Goldblatt) hypertension in the dog and the similar condition of essential hypertension in the human have in the main yielded negative results. Thus attempts to demonstrate the presence of the pressor principle of the posterior pituitary in the blood of hypertensive dogs and in the blood, spinal fluid, and urine of hypertensive humans have been largely unsuccessful. Moreover, total hypophysectomy did not interfere with the development of hypertension following bilateral renal artery constriction in dogs (1, 2), and repeated injections of solution of posterior pituitary did not cause a further increase in the blood pressure of renal hypertensive dogs (3). On the other hand, Griffith et al. (4) reported the production of hypertension in rats by injections of vasopressin and Sattler and Ingram (5) found that injury to the supraopticohypophysial system in dogs with experimental renal hypertension produced significant reductions in blood pressure. The hypothesis that essential hypertension in the human is due to hypersecretion of the pressor substance of the posterior lobe of the pituitary gland was especially championed by Cushing (6).

In order to elucidate this problem further, we have studied the activity of the posterior pituitary in renal hypertensive dogs by assaying their excretion of the urinary antidiuretic principle during normal hydration and during dehydration. Most authorities agree that the urinary antidiuretic substance of the normotensive dog is secreted by the posterior pituitary and that the secretion varies more or less inversely with the state of hydration.

METHODS. Eight dogs were used; four before and after the production of hypertension by the method of Goldblatt (7), two only during normotension, and two only during hypertension. Mean blood pressure readings were obtained by puncture of a femoral artery two or three times a week. Blood urea nitrogen studies, urinalyses, and body weight determinations were made at monthly or bimonthly intervals.

Determinations of the excretion of the urinary antidiuretic principle during normal hydration and during dehydration were made at monthly intervals while the dogs were normotensive and at monthly or bimonthly intervals after hypertension was produced. Four determinations were made during normotension and four during hypertension on each dog except as indicated above. For this purpose one specimen of urine was collected in a metabolism cage during twenty-four hours of normal hydration and a second specimen during a subsequent

[1] This work was aided by a grant from the Graduate School Research Fund of the University of Illinois.

TABLE 1

Excretion of urinary antidiuretic principle in normotensive and renal hypertensive dogs during normal hydration and during dehydration (rat method of Burn (8))

DOG NO.		NORMOTENSION				HYPERTENSION			
		No. of determinations	Time to maximum (urinary) excretion: Range	Time to maximum (urinary) excretion: Average	BP range	No. of determinations	Time to maximum (urinary) excretion: Range	Time to maximum (urinary) excretion: Average	BP range
			min.	*min.*	*mm. Hg*		*min.*	*min.*	*mm. Hg*
1	NH	4	85–90	88	122–132	4	85–92	88	160–180
	D	**4**	**119–130**	**123**		**4**	**110–115**	**111**	
2	NH	4	83–86	85	106–118	4	83–90	87	160–170
	D	**4**	**99–110**	**102**		**4**	**107–115**	**111**	
3	NH	4	85–92	88	130–150	4	83–90	87	150–168
	D	**4**	**105–110**	**107**		**4**	**108–116**	**111**	
4	NH	4	84–90	87	115–125	4	88–95	90	148–160
	D	**4**	**105–110**	**107**		**4**	**108–116**	**112**	
5	NH	4	85–89	86	128–136				
	D	**4**	**106–112**	**108**					
6	NH	4	86–88	87	140–150				
	D	**4**	**103–107**	**105**					
7	NH					4	84–88	87	162–184
						4	**103–110**	**106**	
8	NH					4	88–90	89	168–180
	D					**4**	**104–112**	**108**	
Grand average..................				87 **109**				88 **110**	

NH = Normal hydration. D = Dehydration. (Normal hydration figures in regular type; dehydration figures in boldface type.)

Controls

	NO. OF DETERMINATIONS	TIME TO MAXIMUM EXCRETION: Range	TIME TO MAXIMUM EXCRETION: Average		NO. OF DETERMINATIONS	TIME TO MAXIMUM EXCRETION: Range	TIME TO MAXIMUM EXCRETION: Average
		min.	*min.*			*min.*	*min.*
Vasopressin 2 mu/100 gm. of rat	6	140–150	144	Vasopressin 8 mu/100 gm. of rat	4	190–200	195
Vasopressin 4 mu/100 gm. of rat	16	160–180	170	Physiological saline 1.5 cc./100 gm. of rat	11	76–86	82

Mu = milliunit.

forty-eight hour period of water deprivation. After filtration, each urine specimen was dialyzed in a cellophane bag against running tap water for three hours, and concentrated to a volume of 8 to 15 cc. at an absolute pressure of 20 to 30 mm. Hg and 35–38°C. Each concentrate was then assayed for antidiuretic potency by injection into a group of four rats, using the method of Burn (8) which involves the determination of the so-called "time to maximum (urinary) excretion." Control assays were conducted with vasopressin (Pitressin)[2] and with physiological salt solution.

Results. The results are summarized in table 1. The dogs remained in excellent condition throughout the study as shown by their appetites, body weights, and normal urinalyses and blood urea nitrogens.

Discussion. The results demonstrate clearly there was no significant difference in the amount of the antidiuretic principle in the urines of the renal hypertensive dogs, as contrasted with the normotensive animals, either during normal hydration or during dehydration. Dehydration produced a like appearance of the principle in experimental renal hypertension as in normotension. The results consequently do not support, but do not rule out, the possibility that the activity of the posterior lobe of the pituitary is altered in experimental renal hypertension.

CONCLUSIONS

1. The excretion of the urinary antidiuretic principle in dogs during normal hydration and during dehydration was not changed by the production of experimental renal (Goldblatt) hypertension.
2. These results do not support, but do not rule out, the possibility of altered posterior pituitary function in experimental renal hypertension in the dog.

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[2] Generously supplied by Dr. Oliver Kamm of Parke, Davis and Company, Detroit, Michigan.

SYMPATHETIC AND VAGAL INTERACTION IN EMOTIONAL RESPONSES OF THE HEART RATE

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This paper reports an attempt to examine more completely than hitherto the activity of the autonomic system, as manifested in changes of cardiac rate, in response to emotional stimulation, and to correlate the data obtained with present knowledge of these systems gathered from more direct examinations.

METHOD. Unanesthetized dogs and cats were used. Heart rates were recorded by a Grass ink-writing galvanometer driven by a resistance-capacity coupled amplifier. The electrodes were of solder. One was placed precordially, the second to the right chest wall, and they were both held in place by an elastic band encircling the chest. When respiration was registered, a small blood-pressure cuff was wrapped around the animal's chest and fixed. Changes of pressure in the cuff were recorded either by a tambour which wrote on a kymograph or by a piezo-electric crystal which was connected to an amplifier and recorded by another pen of the ink-writer.

The animal under observation was placed in a cage, and the leads for the electrocardiogram and respiration were led out the top. The cage was large enough to allow the animal considerable freedom of movement.

After the animal was placed in the cage an interval (30 min. to 2 hrs.) was allowed for a constant slow heart rate to obtain. Great care was needed, especially with dogs, to exclude extraneous noises; and quiet was maintained inside the room. The ink-writer was usually enclosed to minimize the noise of its motor. Stimulation was produced by the noise made by hitting a table top with an iron rod 3 or 4 times in less than 2 sec. or by a pistol shot. The animal was unable to see the observer at any time and in either form of excitation no warning was given.

Observations were made on intact animals and on the same animals after various extirpating operations had been performed. The operations were performed aseptically under ether anesthesia with artificial respiration. A period of a week was allowed to elapse between operations when more than one was performed on a single animal. Observations were made usually not sooner than 24 to 48 hrs. after operation, nor later than 1 month after the first operation. The latter precautions were taken to exclude possible post-operative depression on the one hand, and regrowth on the other. It was soon found that the form of response was remarkably similar in different animals and repeatable in the same animal, so that the observations made on any one subject were few in order to avoid the complication of conditioning.

[1] Fellow of the Rockefeller Foundation.

To obtain accurate graphical representation of the changes in cardiac rate and rhythm, the rate per minute was calculated for each R-R interval in the electrocardiogram and was plotted against the time corresponding to the middle of that interval. The plotting was done on a semi-logarithmic scale in order to show percentile and absolute values.

When it was desirable to show long-range changes in rate, the number of beats per 10 sec. was converted into number of beats per minute and plotted against the time corresponding to the end of the 10 sec. period.

Results. When an unexpected noise was made the animals gave a quick start, took several breaths, or changed position. Dogs often got up if lying down, whereas the cats rarely did.

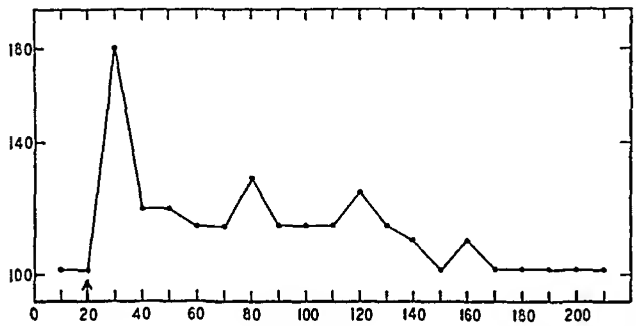

Fig. 1

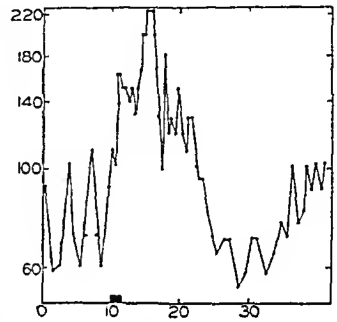

Fig. 2

Fig. 1. A typical response of the heart rate of a dog to unexpected stimulation (at arrow). Ordinates: heart rate per min. calculated from beats per 10 sec. (dots), logarithmic scale. Abscissae: time in seconds.

Fig. 2. The early part of a response of the heart rate of a dog in detail. In this and all following similar figures the rate per minute for each beat has been calculated and plotted (dots) with ordinates: number of beats per minute, logarithmic scale; abscissae, time in seconds; heavy black line, period of stimulation.

A. *Normal animals.* Intact dogs gave a remarkably constant response to a startling stimulation. Variations were small, and when they occurred usually were repeatedly demonstrable for the individual. The response began within one-fifth of a second or less with a loss of the respiratory rhythm of the pulse and an increasing heart rate, which progressed, with interruptions, to a maximum reached in 3 to 4 sec. The rate of individual beats at this peak was 225 per min. or over and was maintained for 4 to 6 sec. At this time the heart rate usually fell toward the control level, sometimes crossing it. Characteristically, this fall was sudden and began with one or more very slow beats, ordinarily lasted about 10 sec., and was succeeded by either a flattening at this level or a significant rise on a slope, more gradual than that of the initial rise which reached a peak, variable in height, at 45 to 60 sec. after the stimulus. From this time onward undulations in the rate, sometimes at quite regular intervals, were common. All evidence of the response was gone usually after 2 to 3 min. but it would occasionally last as long as 7 min. (see figs. 1 and 2).

A not infrequent variation from this picture was the addition of several beats at individual rates up to 300 per min. in the first 2 sec. after stimulation. These beats were followed by a slight fall and then a gradual rise along the curve just described

In normal dogs the resting heart rates varied from 100 down to approximately 60 per min. A marked variation with respiration was usually present when the rate of the pulse and of the respiration was slow. Within the limits just mentioned the resting level of the cardiac rate seemed to have very little influence on the height of the initial response. However, the fall secondary to the initial rise was usually more noticeable, but quantitatively no greater, in the animals with a higher rate and no variation with respiration during the control period, than in the others (cf. figs. 2 and 5). When a variation with respiration was present in the control period, the rate of the slowest beats during the fall usually corresponded closely to that of the slowest beats of the control period (fig. 2). The determination of the cardiac rhythm by the respiration, however, is not dependent upon the rate of the respiration and of the pulse alone (p. 477). Frequently, a change in respiratory cardiac rhythm was the first evidence of response and the rhythm often returned to the degree of influence present during the control period after the cardiac rate had reached its control level. Exceptionally, the sinus arrhythmia could be maintained throughout the response with the rates of individual beats being as high as 200 per min. (fig. 3).

What has been described for the reaction of dogs can, in large measure, be said for cats. Quantitatively, the height of response was similar in the two species, and members of each group showed a few very rapid beats at the beginning of the reaction. Variability was more marked with cats and was seen not only in the group as a whole, but in the same individual from time to time. A frequent deviation from the irregular response shown by dogs was a smoother curve, quite similar to that of dogs with the vagi cut (see p. 471, and fig. 4). The initial increase in rate following stimulation often terminated in 5 sec. to be followed successively by a slight fall and a peak, higher than the first, occurring at 15 to 30 sec. The fall in rate, secondary to the initial rise, so typical of the dogs, was not so profound in cats, and in the series never crossed the control level. Whereas the reaction of cats could be indistinguishable from that of dogs in the first 10 sec., the later part of the response often showed quite variable changes in rate that could go to very high levels. These second rises in rate tended to come earlier than in dogs; i.e., in 20 to 40 sec. rather than in 45 to 60 sec.

The first 25 sec. of an unusual response in a normal cat is shown in figure 3. Immediately after the stimulus the record was spoiled by movement artifact. However, the mechanism of slowing by increasingly longer intervals between beats during expiration, with the maintenance of very short beats during inspiration, is well shown.

B. *The influence of adrenaline.* Adrenaline was excluded from the circulation by removal of one adrenal and denervation of the other. When this was done, the only change detectable in the response of either the dog or the cat was an abolition of, or a marked decrease in, the magnitude of the late accelerations.

The heart of one cat was denervated to act as an indicator for the appearance of adrenaline. This cat was startled repeatedly. The maximal acceleration obtained was a gradual increase of 30 beats per min., which began at 14 sec. after stimulation and was maintained on a constant plateau for 4 min. In no instance was there any evidence of an increase in rate occurring before 12 sec. from the stimulus.

C. *Accelerator activity.* Dogs with the vagi cut in the neck and adrenaline excluded, were subjected to experiment 24 to 48 hrs. after operation. It was necessary in these animals to punch the vocal cords to permit adequate respiration. The resting level of the pulse in such animals was 120 to 130 beats per min., and the rhythm was quite regular.

The response to a stimulus was prompt, the heart rate rising perceptibly within 1 to 2 sec.; i.e., within the first 1 to 3 beats. The rate rose on a smooth, steep

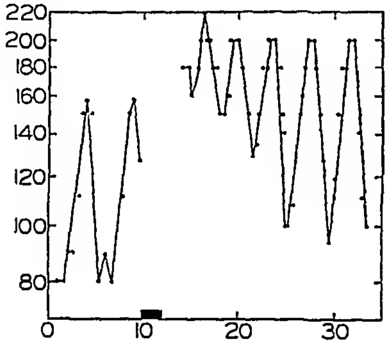

Fig. 3

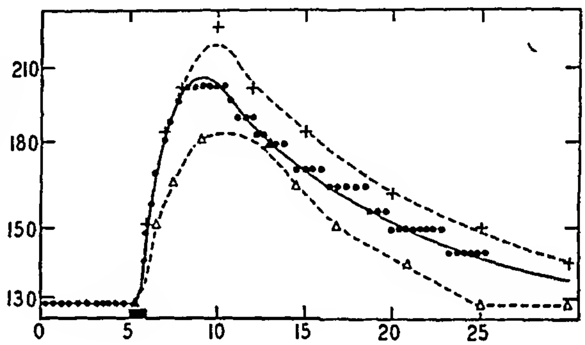

Fig. 4

Fig. 3. The early part of an unusual response in a normal cat. Note the maintenance of respiratory arrhythmia and the slowing effected by increasingly longer beats in expiration.

Fig. 4. Accelerator response. Three experiments from two dogs with vagi cut and adrenaline excluded. In the middle curve each beat is represented by a dot. The steppage effect is due mainly to an artifact introduced in the measurement. Only key points are plotted in the other two curves. Note total lack of respiratory arrhythmia.

slope to reach, in 3 to 5 sec., a maximum which was maintained for 3 to 4 sec. The rate then fell on almost a straight line, returning to the initial level in 20 to 25 sec. from the time of stimulation. Figure 4 shows three experiments on two different animals. Figure 5 show one of the curves superimposed upon the pattern obtained in the same animal before any operation.

No similar group of experiments was done on cats with the vagi severed, because the resting heart in the animals so prepared was too rapid (over 200 per min.) to allow an adequate response.

D. *Vagal activity.* Dogs were deprived of their cardiac sympathetic supply by removal of the stellate ganglia and the thoracic sympathetic chains as far down as the 6th rib. Adrenaline was excluded from the reaction.

The most striking changes in the records of these animals after the operation were the somewhat slower basal rate (48 to 60 beats per min.) and the greatly increased prominence of the respiratory influence on the cardiac rhythm.

These animals also reacted to the startle very promptly—usually a change could be detected within the first beat. In the more usual response the pulse rate did not rise above the rate of a denervated heart; i.e., 100 to 110 beats per min. This rate usually was only slightly higher than that of the fastest beats of the control period (fig. 6). By far the most striking feature of these animals was a change in cardiac rhythm. There was usually a complete loss of the slower beats occurring in late expiration, and it was this loss that in great measure was responsible for the increase in rate.

In a more exceptional response, typical only of a few individuals, the first sign of reaction was 4 to 5 beats of very high rate (200 to 350 per min.) occurring immediately (0.2 sec.) after the startle. These fast beats were then followed by

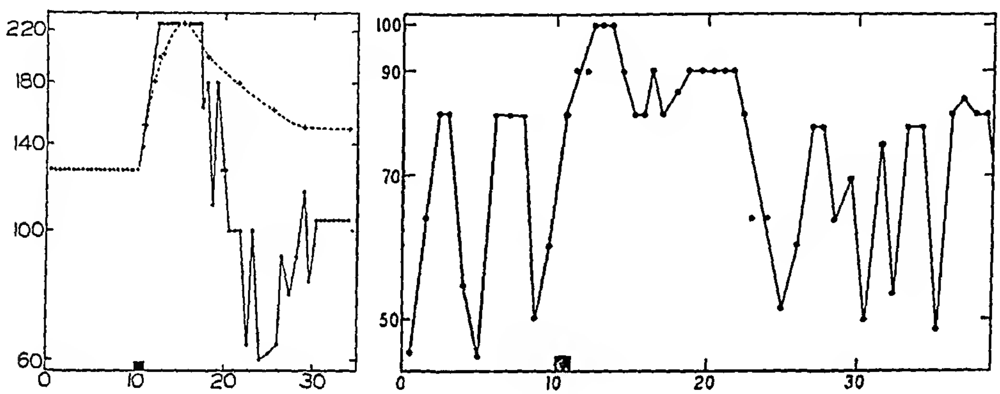

Fig. 5 Fig. 6

Fig. 5. Rôle of vagi in secondary slowing. Dots and solid line represent response of a normal dog. Crosses and dash line show the accelerator response in the same dog after severance of the vagi. Note that the control period showed the same pulse rate in both instances and no respiratory arrhythmia.

Fig. 6. Inhibition of the vagus. The response of the dog after removal of the sympathetic accelerators and carotid sinuses bilaterally. Note pronounced respiratory arrhythmia in control period. This response is similar to that of a dog with sympathetic accelerators out but with carotid sinuses not removed. Each beat is plotted.

the course of the response just described. The long intervals occasionally observed early in the course of the response were not seen, however, if these rapid beats were not present (fig. 7).

The response lasted 8 to 15 sec. and showed a very abrupt end, usually being initiated by one very long interval between beats. This interval was sometimes succeeded by similar long intervals of somewhat shorter duration leading to a resumption of normal rate and rhythm. At other times there was a more gradual decline of the response to normal levels, after which normal rhythm returned. In one dog, despite the maintenance of the threatening stimulus for 16 sec., the heart rate began to decline in 12 sec. and normal rhythm was resumed in 25.

In cats with the sympathetic supply to the heart extirpated the response in general shape and time course was quite similar to that of dogs. Fewer irregularities occurred in the curve, however, and, also, several animals on occasion

showed increases of rate well above that of the denervated heart; i.e., up to 150 to 160 beats per min. for 3 to 5 sec. When these animals were presented to a barking dog their heart rates rose to very high levels, 200 or over, despite the inactivation of the adrenals. This rise was prompt, showed a quick decline, and was of such magnitude that it is very unlikely that sympathin could have been the responsible agent (Partington, 1936).

E. *The influence of the carotid sinus.* The carotid sinus was resected bilaterally after the method of Heymans (1933). When this was done in an otherwise normal dog the resting level of the pulse rose from 60 to 100 beats per min., and the rhythm was entirely regular. The response to the unexpected noise was now a smooth curve much like that seen in the group of animals having only the accelerators present (fig. 4). Instead of the profound secondary fall from 150

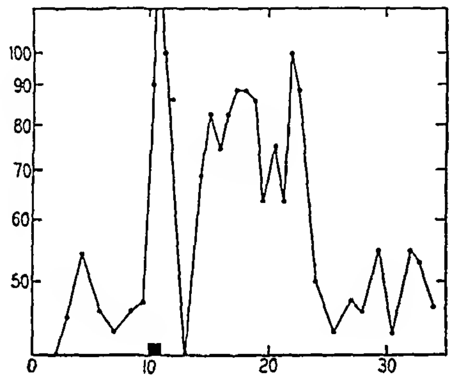

Fig. 7

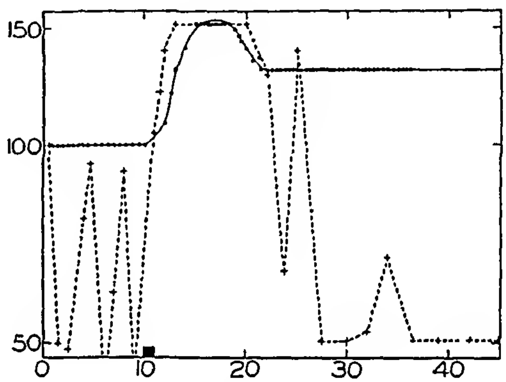

Fig. 8

Fig. 7. Several fast beats of vagal origin seen in a dog with vagi intact but with sympathetic accelerators removed and adrenaline excluded. The beats went off the chart to a rate of 180 per min. Note the profound fall secondary to these fast beats and the sudden termination of the response. Each beat is plotted.

Fig. 8. The effect of removal of the carotid sinuses when the sympathetic accelerators are intact. Crosses and dash line show pattern of normal response (key points only). Dots and solid line show response in same dog after removal of carotid sinuses (all beats).

to 54 beats per min., seen in the control observations on this animal, the heart rate stayed well above the resting level for several minutes and showed no irregularities. The very long duration of this response is attributed in part to the action of adrenaline. It is interesting that the height of the response was identical with that seen in the control observation despite the difference in resting pulse levels (fig. 8).

When the carotid sinus was removed from an animal previously deprived of its sympathetic supply to the heart and with its adrenals excluded, no such dramatic change appeared. The respiratory cycle of the cardiac rhythm was most pronounced, and the duration and shape of the response was identical with that of an animal with the carotid sinuses present (fig. 6).

F. *Influence of respiration.* When either a cat or a dog is startled the first respiratory change is 3 to 5 deep, rapid breaths. These may be followed by

either a continuance of the deep breathing or a period of apnea. Figure 9 illustrates the respiratory change of the latter type in a dog deprived of its sympathetic cardiac supply in response to a very slight noise. It would seem from this record that the corresponding increase in cardiac rate is due to the loss of the slow beats normally occurring in expiration, as no single interval between beats is shorter than those of the control period. This, then, would indicate that the heart was not truly accelerated but that the change in rhythm necessitated by the change in respiration was the sole cause of the faster pulse. On the other hand, a marked deceleration of the heart occurred in an intact cat subsequent to an initial high rise, in the second 10 sec. period following the stimulus. This period of slowing corresponded exactly to a period of apnea. When respiration was again resumed the pulse rate immediately increased.

If the respiratory rhythm, imposed upon the cardiac rhythm, is maintained throughout the response, it plays a very important rôle in shaping the pattern (p. 470 and fig. 3).

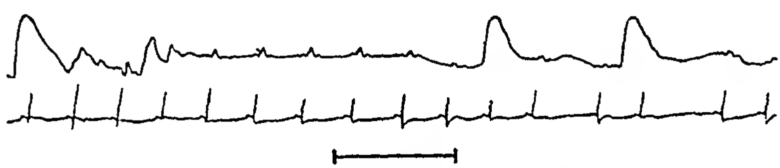

Fig. 9. Cardiac acceleration due to apnea seen in the same dog as in figure 6. Top tracing shows respiration (inspiration up); bottom record, electrocardiogram. The normal rate and rhythm of the heart and respiration are resumed at the right of the record. The acceleration of the heart during the period of apnea has been accomplished solely by the dropping of long intervals. Heavy black line indicates 1 sec.

That the interrelationship of cardiac and respiratory rhythms is complex is shown by the following observations. A dog, deprived of its sympathetics to the heart and its carotid sinus and depressor nerves, showed periods of panting that left the heart rate unaltered at 114 per min. Also, a normal dog which after a startle showed a rise in pulse rate followed by a profound fall to 54 per min., failed to exhibit any sinus arrhythmia despite the maintenance of a uniform respiration of 18 per min. at this time (see normal curve in fig. 8). On the other hand, long intervals between beats, interrupting the course or bringing to sudden termination the faster rate in intact animals and also in those with only the vagus remaining, coincide with the expiratory phase of a deep respiration.

Discussion. From the foregoing data it is concluded that the sympathetic accelerators to the heart are largely responsible for the magnitude of the cardiac acceleration subsequent to brief unexpected stimulation (cf. figs. 4 and 6). Using a technique of stimulation similar to the one presented in this paper, Beebe-Center and Stevens (1937, 1938) have described the reaction of the heart rate of intact cats and a gun-shy dog. They noted the rapidity with which the response appeared and the presence of occasional decelerating vagal activity in its course. The major part of the cardiac acceleration, however, they attributed to inhibition of vagal influence and felt that the sympathetics played only a minor

rôle. They performed no extirpations. Likewise, Whitehorn, Kaufman and Thomas (1935) and Bazett (1941) state that the sympathetic system is too slow in its action to account for rapid changes in rate. In contrast to these statements, the observations described on p. 471 and in figure 4 indicate that the sympathetics are capable of effecting changes easily detectable within 0.5 sec. in many instances. The positive accelerations shown by the progressing decrease in the interval between beats stop in these experiments in from 3 to 7 sec. This time, then, forms a limit within which it is probable that the heightened discharge of the sympathetic fibres ceases.

Rosenblueth and Simeone (1934) have described curves obtained from direct stimulation (for a 10 sec. period at a frequency of 8 per sec.) of the accelerators to the cat heart, which was isolated from the central nervous system. The shapes of the curves are identical with those shown in figure 4. Likewise, Morison (1935) has published curves of similar shape for the reflex speeding of the heart when the vagi are cut. However, the time parameters are at variance in that the curves obtained in response to unexpected stimulation are very much steeper in their initial ascent and return to the resting level more promptly than do the curves obtained from direct or reflex stimulation. Because the shape of the curves obtained from the dogs coincides so well with those obtained by these authors in cats, it is reasonable to infer a similarity of process in the two species.

The assumption that the response of the accelerators after an animal is startled is a brief burst of impulses of high frequency (20 to 30 per sec., see Rosenblueth, 1932) lasting less than 3 to 7 sec., would account for the discrepancy in the time of ascent to high rates between the curves reported here and those described by these other authors. Figure 4 indicates a grading of response which could be explained on the basis of change in either frequency or duration of accelerator discharge; it is similar to a figure reported by Morison in which high frequency of afferent stimulation evoked larger and more lasting reflex acceleration than did low.

The fact that the descent of the curves in figure 4 is steeper than that established by Rosenblueth and Simeone may be due to the carotid sinuses. Pitts, Larrabee and Bronk (1941) have shown, by direct recording, that stimulation of the carotid sinus nerves inhibits the discharge of accelerator impulses. It is interesting, however, that in these experiments by far the greatest factor causing slowing was the vagi, and if they were cut no slowing beyond the initial resting level took place.

Rosenblueth and Simeone have shown that the vagus and accelerators work independently if the effects of either are gauged by the percentile change in heart rate effected by direct stimulation at known frequencies. Likewise, it can be said that in the response to the startling stimulus, the vagus, in the main, acts upon the background offered by the change in rate effected by the accelerators and that its activity is, in part, determined by that background.

This mechanism is well illustrated by the observation that the profound fall in cardiac rate subsequent to an initial rise, more pronounced in dogs (fig. 5), was never seen either in animals deprived of the sympathetics or in those with vagi

cut. It seems probable, therefore, that this slowing is dependent upon the integrity of the vagi, and also upon the attainment of a critical level, either of pulse rate or of blood pressure, which is responsible for tripping a depressor mechanism which acts primarily through the vagi. That the carotid sinus is the initiating factor is implied by the experiment described on page 473 (fig. 8). It is noteworthy that this slowing of the pulse occurs at the time when the speeding of the denervated heart by adrenaline begins, and reaches its maximum: i.e., in 10 to 20 sec. Although no quantitative analysis was attempted the point was clear that those intact animals that responded by a high and sustained rise in pulse rate showed the most pronounced subsequent slowing. Confirmatory evidence may also be derived from the observation that when the respiratory rhythm of the heart mediated through the vagi, was kept throughout the response, it became much more marked when the pulse rate rose to high levels (fig. 3, and see below).

However, there is a degree of reciprocal innervation shown by the vagi that is not covered by what has just been said. There seems to be some accelerating mechanism in the vagus, common to both cats and dogs, that is responsible for the few very rapid beats seen in the first 1 or 2 sec. after stimulation (page 472 and fig. 7). Some of these beats have an ectopic origin as the electrocardiogram shows inverted P or QRS waves. Not all of them fall into this category, however, and because they are few, come so promptly, and are at such a fast rate,it would be an error to classify them as being due to the vagal accelerators described by Jourdan and Nowak (1934).

In dogs deprived of the accelerators and with the effect of adrenaline excluded there is a speeding of the heart upon stimulation which, except for the few fast beats just described, never exceeds the rate of the denervated heart, and, therefore, can be explained solely by the inhibition of vagal tone. This conclusion is in agreement with that of Bouckaert and Heymans (1936), but it is in disagreement with the statement of Brouha, Cannon and Dill (1936) that there is a high degree of cardiac acceleration following slight emotional stimulation in dogs deprived of the sympathetic chains from the stellate to the sacral ganglia.

The inhibition of vagal tone just referred to allows full play to the sympathetic accelerators, but accounts for very little of the acceleration seen in the intact animal.

Cats show little secondary fall in pulse rate in comparison to dogs, and intact animals often yield curves that closely approach those of pure sympathetic activity. This species predominance of the sympathetic is in accord with the report of Bender (1938) and with experience of others working on totally sympathectomized cats and dogs.

That there are important cardio-accelerator fibres in the vagi of cats is shown by the evidence given on p. 472. The data presented throw no light on their nature or the mode of their activity. The time course of their effects, however, coincides with that of both accelerator action and vagal inhibition already described.

Respiratory influences, mediated by the vagi, are of importance in the control of rate and rhythm and are one of the most important sources of decelerating

vagal action. Anrep, Pascual and Rössler (1936), using the innervated heart-lung preparation, have shown that the respiratory cardiac rhythm is of extremely complex origin having many central and reflex components. Generally speaking, those influences which raise vagal tone tend to increase the prominence of the respiratory rhythm, while influences which lower vagal tone decrease it. Anrep et al. thus account for the lack of respiratory arrhythmia in a dog without depressor nerves and carotid sinuses as due to a lowering of vagal tone. This is, of course, correct if it is kept in mind that the question is one not only of the absolute vagal tone but of that value relative to the tone of the accelerators. There may be no detectable respiratory arrythmia in a dog deprived only of its carotid sinuses, figure 8, but after removal of the sympathetics that arrhythmia becomes even more pronounced than in the normal (fig. 6).

Anrep and his collaborators have shown that it is mainly the inhibition of vagal tone in early inspiration that accounts for speeding the heart at that time. They state, further, that large doses of morphine prevent respiratory arrhythmia by greatly strengthening vagal tone, so that a uniform, very slow pulse results. This is analogous to the slowing of the pulse without arrhythmia that occasionally takes place in the period of secondary fall when the respiration is uniform and slow.

Apnea alone may be responsible for either a slowing or a speeding of the heart rate, depending in part upon the rate at the time. Because apnea, presumably secondary to hyperventilation, may occur at the end of the 10 sec. period following stimulation, it may account for some of the slowing seen in this period. This is particularly true in cats in which the carotid sinus mechanism is not so prominent as in dogs. In dogs, however, the carotid sinus may be very largely responsible for the cardiac slowing and the apnea.

The secondary rises in heart rate occurring in dogs after 45 sec. and in cats usually after 30 sec. are attributed to a release of the depressing mechanism, and to the influence of adrenaline which at this time is well established. The distinct impression is gained that adrenaline plays a more prominent rôle in cats than in dogs, as cats are more likely to give high peaks at this time. Despite marked rises in cardiac rate, especially in cats, there may be a return to normal in 40 to 50 sec., which is not interrupted by secondary accelerations. This raises the question as to whether adrenaline was secreted in any significant amounts in spite of marked sympathetic activity of the cardio-accelerators. Although the same kind of response was occasionally seen in dogs, it was more rare probably because the carotid sinus is more active in this species, as is shown by late rises of small degree despite the exclusion of the adrenals. The undulations that often take place after 60 sec. are interpreted as being due to overswinging of the pressor receptors.

SUMMARY

Changes in heart rate of unanesthetized dogs and cats, startled by a short, unexpected noise, were recorded electrically. The cardiac responses from the animals when normal were compared to the responses from the same animals after various nerves had been cut.

Intact dogs and cats yielded a complex pattern of a sudden, high rise in heart rate, beginning immediately after the startle. This was successively followed by a sharp fall, more pronounced in dogs, a second rise of variable height, and thereafter, several undulations in rate until a termination of the response in 2–3 min. (p. 469 and figs. 1, 2, 5).

Adrenaline plays a more prominent rôle in cats than in dogs; but its action in either species appears only after 12 sec.

Dogs in which the vagi and depressors were cut and adrenaline excluded, showed pure accelerator activity. The response was similar to that of the normal in promptness and magnitude, but was simpler with no secondary fall or further undulations (p. 471 and figs. 4 and 5).

In dogs and cats with the sympathetic cardio-accelerators removed and with adrenaline excluded, startle was promptly followed by inhibition of vagal tone (figs. 6 and 7). Cats in addition showed an acceleration that was greater than could be accounted for by loss of vagal tonic influence alone (p. 472 and p. 473). Unless there were a few rapid beats of vagal origin occurring in certain individuals immediately after the stimulus, no fall in rate subsequent to the initial rise was seen. If these rapid beats were present they were commonly followed by one very slow beat only. This seems to indicate that usually the increase of heart rate did not raise arterial pressure to a degree sufficient to trip a depressor mechanism acting primarily through the vagus. Evidence is presented that the carotid sinus is involved (p. 473 and figs. 6 and 8).

The effect of respiration on cardiac rhythm is complex and may greatly affect the pattern of response (fig. 3). Apnea may cause a speeding of the heart (fig. 9) or may be accompanied by a slowing (p. 474).

The discussion deals with the quick activity of the sympathetics, the rôle of the vagus, and with the relation of the responses reported here to those obtained by others from direct and reflex cardiac acceleration.

I wish to thank Dr. Walter B. Cannon for much assistance in this work and for his many constructive suggestions.

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EFFECT OF SEX HORMONES ON THE ERYTHROCYTE NUMBER IN THE BLOOD OF THE DOMESTIC FOWL[1]

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A sex difference in the number of erythrocytes in the blood of the domestic fowl was first recorded by Blacher (1, 2), and has since been confirmed by several workers (6, 11, 13, 14). Similar differences have been reported for the dove and pigeon (17) and for a number of wild American birds (16). In immature fowls Chaudhuri (6) reported that the erythrocyte number was intermediate between that found in adult males and females. Juhn and Domm (13) also observed a similarity in the number of red blood cells in immature males and females up to the sixth month after hatching, at which time the number began to increase in males, although the juvenile level was retained by females. This difference was definitely established when the fowls reached sexual maturity at or before nine months of age.

The belief that this difference is due to hormone control has been strengthened by the lowered red cell count found in castrated fowls (1, 2, 12), and by the higher erythrocyte number in sinistrally ovariectomized poulards which have developed ovotestes, and in bilaterally ovariectomized poulards with testis grafts (12).

Recently it has been reported that various estrogens injected into dogs, monkeys, and rats caused a lowered red blood cell count while injections of testosterone propionate effected an increased number (5, 7, 19, 20, 21). The latter was also found to be true when hypogonadic men were treated with testosterone propionate (15).

A study of the effects of androgens and estrogens on the erythrocyte numbers in normal roosters, capons and poulards was undertaken in this laboratory in connection with other observations on the effects of these hormones on secondary sexual characters and behavior. The investigation was extended to include a group of young fowls treated with pregnant mare serum gonadotropin and two groups of intersexual males. A preliminary report of the results has already been published (18).

Materials and methods. Blood was obtained from the wing vein, and diluted with 1.5 per cent solution of sodium citrate in 0.85 per cent sodium chloride.

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All counts were made using an improved hemacytometer with a double Neubauer counting chamber.

All the birds used in these experiments were of the single comb light brown Leghorn variety. Uninjected males, females, capons and sinistrally and bilaterally ovariectomized poulards were used as controls. With the exception of the poulards, which were three years old, all adult birds used were one year old.

Six capons, three bilaterally ovariectomized poulards, and three sinistrally ovariectomized poulards received daily intramuscular injections of testosterone propionate. In a similar manner, eleven capons, three bilaterally ovariectomized poulards and two adult males, received alpha-estradiol benzoate or diethylstilbestrol. The amount of hormone given in each case and the duration of the experiment are recorded in tables 2 and 4.

The results obtained from estrogen and androgen injections suggested that an examination be made of immature chicks injected with a gonadotropin. Consequently counts were made on six males and six females which had received daily injections of pregnant mare serum gonadotropin (table 3).

TABLE 1

Erythrocyte counts in uninjected birds

SEX	NO. OF BIRDS	AVERAGE RED CELL COUNT*	RANGE	STANDARD DEVIATION
Male	18	3.60	2.88–4.27	0.361
Female	18	2.70	2.14–3.15	0.312
Capon	18	2.51	2.07–3.10	0.288
Sinistral poulard	12	2.78	2.41–3.34	0.278
Bilateral poulard	3	2.59	2.46–2.71	0.102

* Expressed in millions per cubic millimeter in all tables.

Counts were also made on two series of intersexual males resulting from the injection of various estrogens during early embryonic life. The first group included thirty-six birds about two years old, while the second group included seventeen birds varying in age from 3 to 5 months. The kinds of hormones used, their concentration and the amounts given are indicated in table 5.

Erythrocyte counts made on three intersexual males and six uninjected poulards during the last stages of molt were compared with those made on the same birds the previous summer prior to the molt.

RESULTS. 1. *Normal males, females, capons and poulards.* The average erythrocyte counts for normal males, females, capons, and poulards are recorded in table 1. In the male the average number of red blood cells per cubic millimeter (3,600,000) is approximately a million higher than in the female (2,700,000). The average erythrocyte numbers of the capon and the bilaterally ovariectomized poulard are below, while that of the sinistrally ovariectomized poulard is above the normal female level.

Assuming sex hormones are the controlling factor in erythrocyte production, these results may be interpreted as follows: The development of an ovotestis in

sinistrally ovariectomized poulards is responsible for the production of androgen causing a rise in blood count. In both capons and bilaterally ovariectomized poulards the erythrocyte picture is the same due to the complete absence of gonads, and therefore sex hormones, in each group. The slightly higher count found in normal females is probably due to the production of a small amount of androgen by the medullary component of the left ovary.

From these observations it would appear that androgens stimulate the cells producing red corpuscles, and that a lack of such hormones causes a decrease in their production, but whether the low count in the female is due to the absence of an adequate amount of androgen, to the neutralizing effect of estrogens, or to a direct inhibitory effect of the female hormone cannot be determined from

TABLE 2

Effect of testosterone propionate on erythrocyte number

BIRD NO.	SEX*	MGM. DAILY	RED CELLS PER CU. MM.		
			11th day	24th day	45 days after injections
1**	C	1.00	3.23†	3.35	2.28‡
2	C	1.25	2.91	3.20	2.41
3**	C	2.00	2.68†	2.85	2.66‡
4	C	2.50	2.06	3.21	
5**	C	3.00	3.30†	3.83	3.16‡
6	C	3.75	2.85	3.37	
7	SP	1.25	3.31	3.67	2.52
8	SP	2.50	3.88	4.37	2.81
9	SP	3.75	2.90	3.27	2.86
10	BP	1.25	2.90	3.40	
11	BP	2.50	2.95	3.64	
12	BP	3.75	2.93	2.97	

* C., capon; SP sinistrally, and BP bilaterally, ovariectomized poulard.

** Counts made on 1, 3, and 5 before injections began were 2,400,000, 2,080,000, and 2,170,000 respectively.

† Counts made on 15th day of injection

‡ Counts made 21 days after injections ceased.

these observations. To shed some light on this problem we injected various sex hormones into normal and operated fowls.

2. *Testosterone injected capons and poulards.* The capons and poulards receiving daily doses of testosterone showed an unmistakable increase in the number of red blood corpuscles after 11 days of injection, and this increase was even more noticeable after twenty-four days (see table 2). In every case, the count made twenty-four days after injections were begun was equal to or approaching that of a normal male. Counts made twenty-one days after cessation of injections had fallen appreciably and were approaching the normal level; while counts made forty-five days after injections had ceased had fallen to the level of uninjected capons and poulards.

There appeared little correlation between the amount of testosterone received and the increment of red cells and no attempt was made to find the threshold or

maximum effect doses, although in both the sinistrally and bilaterally ovariectomized poulards a daily dose of 2.5 mgm. seemed to evoke the greatest increment. Further experiments are necessary to prove whether this is approaching the maximum response, or whether the results are merely coincidental. The latter would appear to be the case since there was no correlation between size of dose and blood count in the six capons.

Since counts in all testosterone-injected birds were higher than in normal ones, it might be expected that gonadotropins injected into immature birds would bring about a rise in the juvenile number of corpuscles due to the androgens precociously produced by the stimulated gonads.

3. *Immature males and females injected with pregnant mare serum gonadotropin.* In the six 29 day old males injected with PMS the average red cell count was 2,760,000 while in the six 32 day old females, similarly treated, the average count was 3,010,000 (see table 3). The four male and six female saline injected controls had average counts of 2,460,000 and 2,660,000 respectively while the normal controls averaged 2,270,000 and 2,480,000. There was very little range

TABLE 3

Effect of PMS gonadotropin on erythrocyte number in juvenile birds

	NO. OF BIRDS	AVERAGE RED CELL COUNT	RANGE	STANDARD DEVIATION
Injected males	6	2.76	2.40–3.36	0.398
Saline injected controls	4	2.46	2.43–3.29	0.125
Normal controls	3	2.27	2.21–2.39	0.083
Injected females	6	3.01	2.71–3.38	0.219
Saline injected controls	6	2.66	2.22–2.98	0.271
Normal controls	8	2.48	2.14–2.75	0.227

Males received 140 I.U. of PMS daily for 24 days and females for 28 days. Injections were begun on the fourth day after hatching.

in the individual birds although the females were consistently slightly higher than the males.

The counts of both saline injected and normal controls were typical of the juvenile level, while those of the treated birds were higher. In the case of the females the average count was well above the normal mature female average, indicating production of androgen by the stimulated juvenile ovary. In addition to a higher blood count, these birds showed evidence of gonad activity in the markedly stimulated comb growth and by crowing, which occurred in males, on the twelfth day of injections. Although this is suggestive evidence a further study of the erythrocyte picture in immature birds stimulated by gonadotropins is desirable.

4. *Estrogen injected capons, poulards and normal males.* The erythrocyte counts of the eleven capons and three bilaterally ovariectomized poulards receiving varying doses of a-estradiol or stilbestrol were, with the exception of capon 3 which had a count of 2,720,000, below the level for a normal female (see

table 4). It was observed in poulard 14, receiving the highest dose of a-estradiol (1.5 mgm.) over a long period of time (43 days), that there was first a fall in the erythrocyte count followed by a rise when the second count was made. In all other cases there was a continued decrease. There appeared no other significant correlation between the dose given and the effect produced. Capons 6 and 8, receiving a-estradiol, showed a greater decrease in red blood cell numbers than capons 9 and 10, receiving the same amounts of stilbestrol for the same length of time. In capon 7 the erythrocyte count returned to the normal capon level when injections were terminated because of sickness of the bird.

The two adult males receiving daily injections of stilbestrol showed a reduction in the number of red corpuscles to almost one-third of the normal male number. It is especially interesting to note that these counts are far below the normal female average. The reason for this is not clear although it has been suggested that it may be due to a greater toxicity of stilbestrol.

TABLE 4

Effect of a-estradiol benzoate and diethylstilbestrol on erythrocyte number

BIRD NO.	SEX*	MGM. DAILY	RED CELLS PER CU. MM.			
			Before	15th day	29th day	43rd day
1	C	0.33 a-estra.	2.36	1.98	2.07**	
2	C	0.33 a-estra.	2.85	2.69	2.22**	
3	C	0.33 a-estra.	2.64	2.72	2.26**	
4	C	0.50 a-estra.			3.65	2.40
5	C	1.00 a-estra.			1.76	1.67
6	C	1.00 a-estra.	3.06	1.90		
7	C	1.50 a-estra.			2.19†	2.57
8	C	1.50 a-estra.	3.40	1.29		
9	C	1.00 stilb.	2.79	2.61		
10	C	1.50 stilb.	2.64	1.51		
11	C	2.00 stilb.	2.74	2.12		
12	BP	0.50 a-estra.			2.30	1.76
13	BP	1.00 a-estra.			1.95	1.37
14	BP	1.50 a-estra.			1.53	1.74
15	R	2.50 stilb.			1.59	1.65
16	R	5.00 stilb.			1.25	1.71

* C., capon; R., rooster; BP., bilaterally ovariectomized poulard.

** Counts made on 25th day of injection, at which time injections were discontinued. Counts 21 days later were 2.53, 3.16, 2.59 respectively, indicating a return to the normal capon level.

† Injections stopped after 21 days because of sickness of bird.

Here it was also observed that in long continued injections (43 days) the initial drop in erythrocyte number was followed by a subsequent rise. The number, however, remained less than half the normal male count. This secondary rise in the number of erythrocytes, also observed in poulard 14, may be due to an adaptation of the individual to the long continued high dosage resulting in a certain degree of tolerance.

Clotting time was prolonged in all estrogen injected birds. In one of the males receiving stilbestrol, it was noticed that a small wound, made by plucking a feather, continued to bleed for several hours.

These observations suggest that estrogens have an inhibitory effect on the production of erythrocytes, and do not produce their effect merely by neutralizing the stimulus initiated by androgens.

From the results obtained from estrogen injections in dogs (20) and in rats (21), it would seem that the lowered red blood cell number found in estrogen-injected individuals and in females can be accounted for by the action of estrogens on the marrow cavities of the long bones. It has been shown that estrogens cause ossification of the bone marrow, which must seriously interfere with erythrocyte production (3, 4, 22).

If this is the cause of the inhibiting effect of female sex hormones, then we may logically assume that the male hormone causes a stimulation of the erythrocyte-

TABLE 5

Erythrocyte counts in adult intersexual males

TREATMENT	INCUBATION DAY INJECTED	NO. OF BIRDS	AVERAGE RED CELL COUNT	RANGE	STANDARD DEVIATION
0.10 mgm. theelin	4	13	2.96	2.44–4.16	0.454*
0.25 mgm. a-estradiol	5	3	2.92	2.82–3.07	0.108
0.50 mgm. a-estradiol	5	12	2.60	1.87–3.18	0.339
2.50 mgm. stilbestrol	7	3	2.61	2.46–2.75	0.118
2000 I.U. estrogen**	4	5	3.21	2.59–3.63	0.347

* High standard deviation due to one bird with the exceptionally high count of 4.16. (See comment on leukosis in discussion, page 485.) Omitting this bird the average count is 2.86 with a standard deviation of 0.271.

** Amniotin, prepared by E. R. Squibb and Sons from pregnant mare's urine.

forming cells in the marrow of the bones. The nature of the stimulus is not known.

5. *Intersexual males.* The results obtained from counts on adult intersexual males, feminized by estrogen injections during early incubation, are shown in table 5. With the exception of only three counts (4,160,000, 3,630,000, and 3,520,000), all counts were lower than those expected for a normal male. No great differences were observed in the effects of theelin, a-estradiol, a non-crystalline estrogenic substance,[2] and stilbestrol, although the average from birds treated with a-estradiol was slightly lower than the others, and that of birds treated with the non-crystalline estrogenic substance slightly higher. These differences, however, are not statistically significant.

Since the condition of the plumage may be used as a quantitative index of estrogen production (8), these birds were arbitrarily classified into four groups based upon the degree of feminization of their plumage (9). I. Males which

[2] Amniotin, prepared by E. R. Squibb and Sons from pregnant mare's urine.

are essentially masculine in general appearance. II. Males which show a prominent scattering of female feathers on hackle, back, and saddle, and a lesser number of scattered female feathers on the breast. III. Males which have a female or nearly female hackle, back, and saddle, while the breast and tail, though predominantly female, still show many scattered male feathers. IV. Males which are practically indistinguishable from the normal female in general appearance.

The average count of the seven class I birds was 2,837,000, while the average of the six class IV birds was 2,730,000. The two intermediate groups had averages of 2,894,000 (7 birds) and 2,978,000 (10 birds) respectively. The bird with the highest blood count was in plumage group 2. There appeared, therefore, to be no correlation between degree of feminization of plumage and degree of erythrocyte reduction. In the case of the three birds receiving stilbestrol on the seventh day of incubation, the blood counts were similar to those of females, although there was apparently no feminization of plumage. Apparently the quantity of estrogen sufficient to inhibit the production of erythrocytes is below that necessary to produce a change in plumage, indicating a lower threshold for the former.

In the three to five-months-old intersexual male birds, no significant differences were observed in the blood counts of the feminized individuals and the controls, although some of the birds showed distinctly feminized plumage. The average number of erythrocytes in 17 treated males was 2,690,000 and in 4 untreated birds 2,580,000, in both cases typical of the normal juvenile male. Since the number of erythrocytes is normally low until after the sixth month, the effect of the estrogen treatment on erythrocyte production should not be apparent until after that time.

6. *Molting birds.* The coincidence of the low counts in the erythrocytes with juvenile molts reported by Juhn and Domm (13) led us to examine the red cell picture in six sinistral poulards and three intersexual males before and during the molting season. In the poulards the average count before molt was 2,830,000 and during molt 2,710,000. In the intersexual males the pre-molt counts averaged 2,830,000 and the molting counts 2,890,000. In the individual birds no consistent change in blood cell count during the molting period was observed. In some cases there was a rise and in others a fall in the number of erythrocytes, but the average difference between the counts was slight. Although molting appeared to have no effect on the red blood counts, the small number of birds examined does not provide adequate evidence for drawing definite conclusions.

During the course of this work it was noticed that occasionally exceptionally high blood counts would occur in a few individuals in a group of fairly uniform birds. This was seen in three intersexual males, having counts of 4,160,000, 3,163,000 and 3,520,000. The possibility that these birds were suffering from some form of the avian leukosis complex was suggested since previous counts on two paralyzed intersexual males were 3,810,000 and 3,680,000, in both cases higher than the normal male level. However, subsequent observations on five

intersexual males, believed to be leukemic, revealed counts above the average in only two cases.

It is known that the group of pathological conditions known collectively as the avian leukosis complex may manifest itself in various ways, affecting the bones, nerves, viscera, or the blood picture. An increased erythrocyte count has not been reported in connection with the disease, although, in erythroblastosis there is a stimulation in the production of immature cells. However, these do not develop into mature erythrocytes consequently an anemic condition results (12). It seems probable therefore that the increased number of mature erythrocytes found in the four birds examined may represent an early phase of the disease, although our evidence is by no means conclusive.

SUMMARY AND CONCLUSIONS

1. Erythrocyte counts were made on normal males, females, capons, and sinistrally and bilaterally ovariectomized poulards. These averages confirm previously published results.
2. The erythrocyte numbers in capons and bilaterally and sinistrally ovariectomized poulards receiving testosterone were above those of uninjected controls. Twenty-one days after injections were discontinued the erythrocyte numbers had fallen and by forty-five days had reached the normal levels of uninjected capons and poulards.
3. Juvenile males and females, injected with pregnant mare serum, had blood counts higher than their controls.
4. The erythrocyte numbers in normal males, capons, and bilaterally ovariectomized poulards receiving a-estradiol or stilbestrol were below those of normal controls; clotting time was prolonged.
5. Adult intersexual males, feminized by injection of estrogens during incubation, showed lowered blood counts, approximating those of normal hens which could not be correlated with the degree of feminization of plumage.
6. Immature intersexual males, feminized by injection of estrogens during incubation, had the same blood counts as controls, although some of the birds showed feminization of plumage.
7. No significant difference in blood counts before and during molt were observed in the nine fowls examined.

In conclusion, it may be stated that testosterone causes a definite increase in the number of erythrocytes in the blood of the fowl while estrogens cause a decrease, the latter effect probably brought about by changes in bone marrow activity. Our evidence, based on counts in intersexual males, seems to show that the threshold for an estrogen effect on the erythrocyte-forming cells is below that of the plumage.

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THIAMINE AND THE SPECIFIC DYNAMIC ACTION OF CARBOHYDRATE AND FAT

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It has been suggested that the specific dynamic action of carbohydrate is due to the heat expended in its intermediary metabolism (see Dann and Chambers, 1930). This intermediary metabolism may result in the formation of glycogen or the production of fat. One would expect that the synthesis of fat from sugar would require more energy than the production of glycogen. Now, since thiamine stimulates new formation of fat (see Whipple, Church and Stevens, 1937; McHenry and Gavin, 1938, 1939), the S.D.A. of glucose should be greater when thiamine is given. In the present study, a comparison has been made between the S.D.A. of glucose given alone, or glucose and thiamine, and of glucose, thiamine and fat. The last mixture was used because it was thought that fat given with glucose plus thiamine would depress the new formation of fat, and therefore, the S.D.A. However, the S.D.A. of fat itself complicates the interpretation of results.

METHOD. The procedure followed was similar to that described in an earlier paper (Ring, 1942). The rats were given the usual diet of Purina Dog Chow. Records of post-absorptive metabolism were obtained for a period of three hours. The rats were then removed from the apparatus and one of the following solutions or mixtures was given by stomach tube:

Three cubic centimeters 50 per cent glucose
Three cubic centimeters 50 per cent glucose plus 50 gamma of thiamine
Three cubic centimeters 50 per cent glucose, 50 gamma of thiamine, $1\frac{1}{2}$ cc. of oleic acid
Three cubic centimeters water plus 50 gamma of thiamine
Three cubic centimeters water, 50 gamma of thiamine, $1\frac{1}{2}$ cc. oleic acid.

After returning the rats to the metabolism apparatus, the oxygen consumption was continuously measured during the succeeding seven hours. Quiet periods were selected each hour and averaged to determine the resting oxygen consumption during this period. The first three solutions were given in rotation at weekly intervals until nine observations had been made. A group of eight rats was kept on this regime. Using a second group, the metabolic effect of the last two mixtures was studied. To estimate the heat production, the respiratory quotients were determined by using the Haldane principle in one quarter of the experiments. Calculations of heat production were based on these figures.

RESULTS. In reporting our findings, the result for each separate experiment is not presented. Only the average increase in metabolism for the three days when the same material was given is to be found in table 1. Thus the grand

average at the bottom of each column and the probable error were calculated from a total of 24 observations. It is clear that the S.D.A. of glucose varies widely in different rats. However, in every rat studied the thiamine increased the metabolic effect of the glucose. This is not due to any marked effect of the thiamine alone on metabolism. The metabolic effect of giving 50 gamma of thiamine dissolved in 3 cc. of water has not been worked out with great care but 22 observations made on 11 rats showed an average increase of 0.9 per cent. Since the difference between the S.D.A. of glucose and of glucose plus thiamine is 3.8 per cent, it seems highly probable that this effect of the thiamine is specifically on the glucose. At any rate, there is less than one chance in 10,000 that the difference of 3.8 per cent is not significant. If this increased S.D.A. is due to the conversion of sugar into fat, then the respiratory quotients should be

TABLE 1

Average percentage increase in metabolism during seven hours after ingestion of food

(Each figure is the average of 3 observations)

RAT NO.	GLUCOSE*	GLUCOSE AND THIAMINE**	GLUCOSE, THIAMINE† AND OLEIC ACID
1	4.8	5.2	2.7
2	8.2	9.4	14.7
3	3.3	9.9	6.4
4	4.5	9.6	5.9
5	1.9	9.0	12.3
6	2.5	10.6	7.0
7	4.9	5.6	2.7
8	3.8	4.6	2.1
Average...........	4.2 ± 0.4	8.0 ± 0.5	6.7 ± 1.0

* Three cubic centimeters 50 per cent glucose.

** Three cubic centimeters 50 per cent glucose plus 50 gamma of thiamine.

† Three cubic centimeter 50 per cent glucose, 50 gamma of thiamine, 1½ cc. oleic acid.

raised by the addition of thiamine to glucose. This was found to be the case. The respiratory quotients during the seven hours after giving glucose without thiamine averaged 0.803 for this group of rats. (It was 0.796 for similar experiments a year ago.) When thiamine was added, the quotient rose to 0.812. When the metabolism was measured for a shorter period of time, the results suggested that thiamine produced its greatest rise in respiratory quotients during the latter half of the seven hour period.

In six of the eight rats studied, the addition of oleic acid to the mixture of glucose and thiamine reduced the S.D.A. Although this reduction is possibly not significant, it is probable from statistical analysis that the addition of fatty acid does not increase the S.D.A. above that of glucose plus thiamine. Yet one would hardly think that the S.D.A. of the fat would be completely absent. Previously, Ring (1942) showed that the S.D.A. of fat plus glucose is greater than that of glucose alone. We, therefore, believe that the addition of fat to the

mixture of glucose and thiamine has depressed the effect of thiamine in stimulating the conversion of sugar into fat. The lower respiratory quotient of 0.797 found after fat was added supports this impression, though other explanations are possible.

It is unlikely that thiamine has any effect on the metabolism of oleic acid. Measurements of the S.D.A. of oleic acid plus thiamine averaged, in eight rats, 6.9 per cent. Previous averages for other rats given oleic acid alone were between 6.6 and 8.7 per cent.

The results obtained support our previous contention (1942) that the S.D.A.s of the various foodstuffs are not additive when two or more are given at the same time. The S.D.A. of fat, glucose and thiamine proved to be 6.7 per cent, or less than half the 14.9 per cent which totaling the individual responses gives. These findings substantiate the theory that the S.D.A. of carbohydrate is due to the energy wasted in intermediary metabolism. That energy is required to convert the foods into a form suitable for storage.

The differences obtained in these experiments are not large and it might be argued that more striking results could have been obtained if the animals studied had been deficient in thiamine. This may be true but such deficient animals have a lowered metabolism (see Göthlin, 1938). Since the thiamine alone would restore the metabolism to normal, it is quite probable that this complication would offset the benefit of studying an increased conversion of carbohydrate into fat.

CONCLUSIONS

1. The specific dynamic effect of glucose (4.2 per cent) is less than that of glucose and thiamine (8.0 per cent) (see table 1).

2. Thiamine in water has very little if any effect upon the basal metabolism (0.9 per cent). When thiamine is added to glucose, the extra energy observed is probably expended in converting the sugar into fat. The fact that the respiratory quotient is higher after giving thiamine and glucose than after glucose alone supports this suggestion.

3. When oleic acid is added to glucose and thiamine, the specific dynamic effect (6.7 per cent) is not increased over that of the glucose and thiamine (8.0 per cent). Unless the oleic acid has no specific dynamic action, the effect of thiamine in stimulating conversion of carbohydrate into fat must be reduced as the lowered respiratory quotient suggests.

4. The specific dynamic effect of oleic acid appears not to be affected by giving thiamine.

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REACTIONS OF THE AORTA IN HEMORRHAGIC HYPOTENSION AND SHOCK[1]

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It is fairly common knowledge among experimentalists that a laboratory procedure designed to induce shock may, for hours, cause only a moderate circulatory imbalance; then quite rapidly blood pressure declines, the heart starts to slow, the pulse pressure decreases and a state or circulatory failure develops which is no longer benefited permanently by transfusions. Such a turning point is generally discernible in hemorrhagic shock which develops after a protracted period of hypotension and subsequent reinfusion of the blood withdrawn (1).

The factors or processes responsible for the phenomenon of irreversibility remain an enigma. Many can be suggested, but none has been incriminated as the only or dominant one.

Since such a circulatory turning point is generally characterized by a marked reduction in pulse pressure, a fairly sudden decrease in cardiac output may be concerned. However, the ratio, systolic discharge/aortic capacity, can be reduced equally by decreasing the numerator or by increasing the denominator. Consequently, it is also conceivable that a fairly sudden enlargement of the aorta due to failure of its intrinsic musculature might account for such pulse pressure changes. Since a number of investigators (for review cf. Bazett (2)) have recently stressed the importance of active changes in aortic capacity and distensibility, and since we (3) unexpectedly found that the aorta decreases in size and increases in extensibility during active hypertension, one of us (4) suggested that "the remote possibility that failure of mechanisms which adapt the size and elasticity of the aorta to changing volumes and pressures of blood may be a decisive factor in circulatory failure needs to be investigated." This communication seeks to report, very briefly, results from three types of experiments which lend *no support* to such a suggestion.

Aortagraph experiments. Eight experiments were performed on anesthetized dogs under mild artificial respiration in the following manner. With the animal on its right side, the left side of the chest was opened by an intercostal incision and forcible retraction of ribs, sufficient to make the upper portion of the ascending aorta accessible. An aortagraph similar to that previously described (3) was stitched to the aorta in line with its diameter and connected by a leak-proof tubing with a Frank segment capsule. The optical record magnified changes approximately 10 to 12 times. An idea of the sensitivity is gained from calculation ($\pi r^2 h$) that in an aorta 30 cm. in length and 1.3 cm. in diameter a change in diameter of 1 mm. (or 1 cm. of record) causes, at normal

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pressures, a volume change of about 5 cc. or 15 per cent of its total volume. Intraaortic pressures were recorded simultaneously with a calibrated optical manometer of the Gregg type.

In general, our experimental plan consisted in reducing arterial pressures to about 50 mm. Hg by fairly rapid bleeding and after maintenance of such pressure for an hour or more to reduce it approximately to 30 mm. for another 45 minutes. Thereupon, all the withdrawn blood was reinfused and the state of progressive circulatory failure awaited.

Our results indicated (1) that during an acute hemorrhage by which mean pressure was reduced from 130–140 to 50–40 mm. Hg, the diastolic and systolic diameters of the aorta diminished directionally as intra-arterial pressures. The form of the pulse also changed with that of the intra-aortic pressure pulse. Since the natural volume elasticity of the aorta was unknown, it was impossible to determine whether such reduction involved more than an elastic retraction. Without this knowledge, continued observations of qualitative directional changes might still have proved useful had it not been for unanticipated difficulties in preventing displacement of such an aortagraph. Our chief difficulty consisted in its dislocation by the assumption of vigorous spontaneous breathing during the low blood pressure period. Obviously, one such displacement during the day's experiment sufficed to alter recorded relationships of aortic size. Consequently, it was necessary to restrict comparisons of aortic diameter/diastolic pressure to certain portions of our experimental day.

During the period of marked hypotension, when processes which later manifest themselves as shock are presumably operating, discordant directional changes in aortic or diastolic pressure never occurred. On reinfusion of the withdrawn blood the diastolic size increased *pari passu* with diastolic pressure and the amplitude of the aortagraph pulses increased with the intra-aortic pulse pressure. In three experiments, however, the aortic diameter decreased a few minutes after blood infusion had stopped, while intra-aortic pressure remained unaltered. This resembled the changes induced by acute hypertension, but was much less in magnitude. It is possible that such effects are mediated without intervention of nerve elements and correspond to the stretch stimulus reactions reported by others (5) in the case of "muscular arteries." During the progressive decline of blood pressure following transfusion, most of our experiments revealed no changes that could not be assigned to passive retraction. In two, however, oscillatory changes of diastolic size and pulse amplitude occurred which were not related to intra-aortic pressure changes.

While such occasional discordances between aortagrams and pressure pulses are perhaps suggestive of occasional active changes in the aorta, their absence in most experiments, their variability and lack of relation to critical periods of circulatory failure prevent us from attaching significance to them as factors influencing the downward course.

Since such studies of the aorta are entirely qualitative and since even a single adjustment during a day's experiment left us without information as to how diastolic diameters corresponded during the initial period of low pressure and

Circumferometer experiments. This procedure consisted in measuring directly, by a specially constructed instrument called a circumferometer, changes in the circumference of a definite region of the descending aorta during different phases of an experiment on hemorrhagic hypotension and shock. In addition to making comparisons of aortic circumference/aortic diastolic pressure at all times, the procedure had the advantage of a measure of comparative quantitation. Thus, by vagal slowing and stoppage of the heart—which lowered pressures from say 100 to 20 mm. Hg—it was possible to construct a control curve of circumference/pressure relations, which could perhaps be assumed to represent passive retraction. However, since Dow and Hamilton (6) presented circumstantial evidence that vagus stimulation may affect elasticity of the aorta by action on its muscular elements, the possibility that it also affects the circumference/pressure relations must be kept in mind. At any event, circumfer-

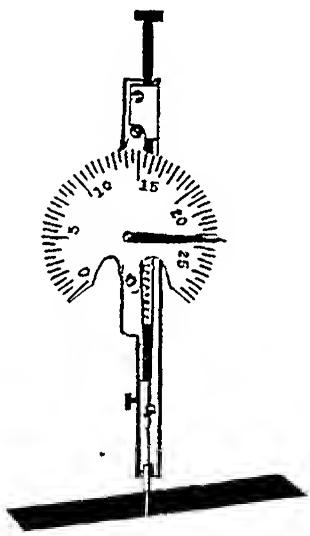

Fig. 1

ence/pressure relations occurring during different phases of the experiment could be compared with a definite curve.

The circumferometer, shown in figure 1, had a fixed and a sliding arm to which an inelastic thread looped around the aorta was attached. The sliding arm operated as a rack geared to a pinion, and to this a pointer was attached. For each measurement, the sliding arm was drawn up until the loop fitted snugly around the aorta during diastole and gave a slight systolic shock to the controlling finger, and the circumference thus determined was read on a dial. Since changes in circumference were approximately 3× as great as changes in diameter and as the scale magnified four times, a total magnification of 12 was realized. However, the readings necessarily depended on personal tactile and visual judgments as to when a "just snug" fit of the encircling thread was achieved. In each instance, a number of measurements were made by each of several experimenters and agreement was usually reached as to the exact measurement.

Measurements of aortic diameter were made frequently throughout six experiments on hemorrhagic shock. In five of these the circumference/pressure

relations during, and immediately after, hemorrhage corresponded rather well with the standard curve derived during vagal inhibition of the heart. In one experiment only, the circumference decreased more. However, neither in this nor in other experiments did we find at any later stage of hemorrhagic hypotension, reinfusion, or final circulatory failure any deviations which were significant enough to indicate that active changes in the aorta participated in process of shock production. Certainly, no sudden or progressive changes were detectable during late periods of severe hypotension when the processes producing irreversibility probably operate to a marked degree. If our method was too crude to detect smaller changes they could not have played a prominent role in producing the characteristic hemodynamic alterations at the crisis. Admittedly, the remote possibility existed that active changes in the aorta were prevented by influence of the previous operative procedures, or that such changes had already taken place before our measurements started.

Pulse conduction rates. To test the foregoing possibility, we studied, in animals submitted to minor operative procedures, only, the changes in the subclavian-femoral conduction rates at equivalent diastolic pressures at various periods of shock experiments. For this purpose we surveyed a large number of records from experiments already reported (1, 7, 8) in which subclavian and femoral pulses had been recorded and in which equivalent diastolic pressures were fortuitously established for brief intervals at different stages of the experiment. In twelve such experiments, measurements failed to reveal significant differences in such conduction times at diverse stages of the experiments; indeed, in half of the records, pulse conduction times were identical.

SUMMARY AND CONCLUSIONS

On the basis of negative evidence from three modes of experimental approach, we are forced to conclude that it appears highly improbable that active changes in the aortic wall play any rôle in the initiation or progression of hemorrhagic shock or in the establishment of an irreversible state.

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CAPILLARY PERMEABILITY TO INTRAVENOUSLY ADMINISTERED GELATINE

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With the renewed interest in gelatine-saline as a possible blood substitute (1, 2), the question of its rate of disappearance from the blood becomes important. In his review of the literature Amberson (3) states that gelatine undoubtedly leaves the blood stream with fair ease. This statement is based upon indirect estimations, made by several investigators, of the concentration of gelatine in blood at varying times following its injection into normal animals.

There are no data on the rate of passage of gelatine through the capillary wall, but one might suppose that it passes into the tissue fluids rather readily in view of its reported rapid disappearance from the blood. We have tested this possibility by determining the relation between the concentrations of gelatine and serum proteins in the blood and in a capillary filtrate obtained by irritation of the intestine by manipulation.

METHOD. Dogs were anesthetized with pentobarbital sodium. The carotid artery was cannulated for recording the mean blood pressure and for bleeding and injections. A mid-line abdominal incision was made through which the small intestine could be withdrawn for manipulation and the collection of fluid.

The animals were bled rapidly a quantity approximating 3 to 4 per cent of their body weight and were immediately transfused with a similar volume of normal saline. The animals were bled again to the same extent and were transfused with cells suspended in a minimum volume of normal saline plus a gelatine-saline solution sufficient to replace the volume of blood withdrawn. The cells were obtained from a donor dog and were washed twice with saline. The gelatine prepared[1] by hydrolysis of alkali-treated bone collagen was given as a 6 or 8 per cent solution in normal saline. In experiment 5 this solution was adjusted to pH 7.3. In experiments 4 and 5 the solution was autoclaved at 15 pounds' pressure for twenty minutes.

Loops of small bowel of varying lengths were removed from the peritoneal cavity, and the serous surface was irritated by pinching, resulting in the flow from the serous surface of fluid presumably derived from injured capillaries. As shown by Beard and Blalock (4) vigorous mechanical manipulation (pinching) results in the production of a fluid having a serum protein concentration equal to that of the blood. This type of irritation was used in all experiments except the first two, in which a milder degree of irritation was produced by

[1] Supplied by Knox Gelatine Co., Johnstown, N. Y. and Camden, N. J. Lot number B 78-1's.

very light pinching following the application of sodium chloride crystals to the gut surface.

Following irritation, the loops of bowel were coiled in a beaker arranged to avoid traction on the mesentery. The beaker and contents were kept warm by an electric lamp. Evaporation was minimized by covering the beaker with a sheet of rubber dam. The first 5 ml. of fluid collected in the beaker were discarded, the surface of the gut was wiped with dry sponges, then 8 to 10 ml. samples of fluid were collected for analyses. Blood samples were taken at the beginning and end of a fluid collection period in most cases, but in some the blood samples were taken before and after two periods of fluid collection or at the middle of a period.

For the determination of gelatine 2 ml. of serum or fluid were diluted with 7 ml. of water and 1 ml. of 50 per cent trichloracetic acid (1) was added. One ml. aliquots of the filtrate were taken for nitrogen determinations. The non-protein nitrogen was determined on 10 ml. aliquots of the supernatant liquid obtained by centrifuging the tungstic acid precipitate from a solution consisting of 4 ml. of serum or fluid, 2 ml. of 10 per cent sodium tungstate, 2 ml. of $\frac{2}{3}$ N sulphuric acid and 32 ml. of water. The total nitrogen was determined on 1 ml. aliquots of a 1:10 dilution of serum or fluid. The procedures of Van Slyke (5) and Van Slyke and Kugel (6) were used for all nitrogen determinations. The usual factor of 6.25 was used to convert nitrogen values into serum protein or gelatine values. All determinations were done in duplicate, and when the variation between duplicates exceeded 3 per cent the determination was repeated.

Results and discussion. The results are summarized in table 1. The figures in the last column indicate the ease with which plasma gelatine passes through the capillary walls relative to the ease with which serum proteins pass through. If both pass through with equal facility the value of $\frac{R_2}{R_1}$ will be unity, but if gelatine passes through less readily the ratio will be less than unity.

It will be seen that the figures in the last column are all less than unity with an average value of 0.58 and with a spread of 0.47–0.64. We interpret this to mean that either all gelatine passes through injured capillary walls at about 0.6 the rate at which serum proteins pass through, or that approximately 35 to 60 per cent of the gelatine consists of particles completely unable to pass through capillaries with the degree of injury encountered in these experiments (see R_2 in table 1). If the first interpretation is correct it is true both when capillary damage is severe enough to allow all plasma proteins to pass through ($R_1 = 1$) and when less severe damage results in only a fractional loss of plasma proteins ($R_1 < 1$), as will be seen by comparing the values for R_1 and R_2/R_1.

We have considered the possibility that the fluid which we obtained is not representative of the capillary filtrate, for during its passage from the capillaries through tissue spaces to the serous surface of the gut the original filtrate might be altered in several ways. First, it might be diluted with preformed tissue fluid containing a relatively high concentration of plasma proteins. Secondly, some of the gelatine might be completely hydrolyzed. Thirdly, a part of the gelatine might be utilized by the local tissues.

In experiment 6, the first fluid sample was collected 2.6 hours after gelatine was injected. The intestinal loops were returned to the peritoneal cavity and vigorously pinched at intervals during the next 3 hours to remove any preformed tissue fluid. A second fluid sample was collected 4 hours after the first. If dilution with preformed tissue fluid is the factor responsible for our results, the second fluid sample should have a value for R_2 at least approaching unity. It will be seen that this is not the case.

TABLE 1

EXPERIMENT NO.	SAMPLES	TIME	SERUM PROTEIN	GELATINE	NON-PROTEIN NITROGEN	SAMPLES USED FOR CALCULATING RATIOS (f: FLUID; s: SERUM)	RATIO OF FLUID PROTEIN TO SERUM PROTEIN CONCENTRATION	RATIO OF FLUID GELATINE TO SERUM GELATINE CONCENTRATION	$\frac{R_2}{R_1}$
		*minutes**	*gram per cent*	*gram per cent*	*mgm. per cent*		R_1	R_2	
1	Serum 1	30	3.43	1.57	27.1				
	Fluid 1	30–46	2.36	0.63	27.2	f_1, s_1	0.69	0.40	0.58
	Serum 2	55	3.41	1.54	28.5				
	Fluid 2	46–55	2.70	0.71	27.2	f_2, s_2	0.79	0.46	0.58
2	Serum 1	35	2.52	1.97	30.1				
	Fluid 1	35–75	2.01	0.86	33.7	f_1, s_1, s_2†	0.85	0.47	0.55
	Serum 2	70	2.21	1.68	35.0				
3	Serum 1	40	1.55	3.09	28.8				
	Fluid 1	40–65	1.58	1.85	27.6	f_1, s_1	1.02	0.60	0.59
	Fluid 2	65–95	1.14	1.49	28.1	f_2, s_2	0.85	0.54	0.64
	Serum 2	95	1.35	2.74	28.8				
	Fluid 3	95–160	1.54	1.33	29.8	f_3, s_3	0.99	0.50	0.51
	Serum 3	160	1.56	2.64	29.7				
4	Serum 1	44	1.71	2.62	27.2				
	Fluid 1	44–90	1.77	1.65	29.5	f_1, s_1, s_2†	1.02	0.65	0.64
	Serum 2	90	1.76	2.49	29.3				
	Fluid 2	92–124	1.89	1.57	31.9	f_2, s_2, s_3†	1.04	0.64	0.62
	Serum 3	124	1.88	2.43	30.1				
5	Serum 1	55	0.93	2.79	35.8				
	Fluid 1	55–65	1.06	1.50	33.5	f_1, s_1	1.14	0.54	0.47
	Fluid 2	65–77	1.09	1.57	33.9	f_2, s_2	1.07	0.62	0.58
	Serum 2	77	1.02	2.53	31.7				
	Fluid 3	104–128	1.00	1.52	36.7	f_3, s_2	0.98	0.60	0.61
	Serum 3	128		2.51	34.8				
6	Serum 1	145	2.15	2.05	26.5				
	Fluid 1	125–180	2.10	1.12	29.1	f_1, s_1	0.98	0.55	0.56
	Fluid 2	372–425	2.76‡	1.19	48.0	f_2, s_2	1.12	0.64	0.57
	Serum 2	402	2.46	1.87	48.6				

* Time represents minutes after injection of gelatine.
† The average of two serum values used.
‡ Considerable hemolysis in sample.

If the gelatine undergoes hydrolysis after the fluid leaves the vascular system, one would expect to find elevated non-protein nitrogen values for the fluid. This is not the case.

It is very unlikely, although difficult to prove, that gelatine would be utilized at a faster rate by the tissues through which the fluid passes than would be the serum proteins which are also present. It would be even more unlikely that the rate of utilization would be so constant from one animal to another.

Therefore the data indicate that none of these possibilities are likely, and that we have, in fact, analyzed a fluid which is probably very nearly identical with the true capillary filtrate.

One must also consider the possibility that the fraction of the plasma gelatine which leaves injured capillaries will also pass through normal capillaries. If this were true one would expect that in those experiments (1 and 2) in which the capillary injury is not sufficient to permit complete passage of serum proteins R_2 would still be equal to R_2 in those experiments (3, 4, 5 and 6) in which serum proteins passed through completely. This is not the case, for in the former experiments the average of R_2 is 0.44 while in the latter the average of R_2 is 0.59.

It is of interest to note that autoclaved gelatine (expts. 4 and 5) does not pass through the walls of the injured capillaries more readily than unautoclaved gelatine (expts. 1, 2, 3 and 6). This is surprising in view of unpublished evidence obtained in this laboratory that autoclaving results in some hydrolysis of the gelatine, as indicated by a 40 per cent increase of its colloid osmotic pressure. One would expect, if the retention of gelatine by capillaries is due to the large size of the particles, that hydrolysis would allow more to escape. Since no more does escape one can perhaps assume either that the hydrolysis involves chiefly particles originally small enough to pass through readily, or that very large particles are split into units still too large to escape.

SUMMARY

It has been shown that intestinal capillaries injured sufficiently to permit the partial or complete passage of serum proteins through their walls allow the passage of only 35 to 60 per cent of plasma gelatine. This is thought to be due either to a slower rate of escape for gelatine than for serum proteins or to the presence of gelatine particles to which the injured capillary is completely impermeable.

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STUDIES IN EXPERIMENTAL TRAUMATIC SHOCK WITH PARTICULAR REFERENCE TO PLASMA POTASSIUM CHANGES[1]

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The research reported here was undertaken in order to investigate the suggestion made by Scudder (12) that potassium might be a toxic factor in shock. A critical examination of much of the data on which this suggestion was based, particularly that dealing with traumatic shock (2, 14), seemed to us to be inconclusive because of the small number of animals used and the variability of the increase in plasma potassium reported. Since one hundred grams of muscle, kidney, liver or brain contain about 400 mgm. of potassium, there is reason to suspect mobilization of a considerable amount of the base from damaged tissue. Winkler, Hoff and Smith (13) showed that 500 mgm. of potassium killed a 10 kgm. dog when injected intravenously over a period of 13 minutes. Furthermore shock has been produced frequently by administration of saline or aqueous extracts of tissues, by tissue autolysates and implants, each of which might contain appreciable amounts of potassium. Recently Pen, Campbell and Manery (11) demonstrated that potassium was the toxic factor in certain alcoholic extracts of muscle which were found to cause death in mice.

We have analysed the plasma in a large series of dogs in which traumatic shock with some hemorrhage was produced by the method employed by Best and Solandt (1). In addition to potassium analyses, measurements of local fluid loss and red cell volume, the water content and chloride concentration of plasma were also estimated. Some sodium analyses were performed, thereby allowing a comparison with the condition of adrenal insufficiency.

In brief, the results demonstrated that the potassium concentration of the plasma increased in a variable manner following muscular trauma. In most cases some decrease in chloride concentration was observed. Although the electrolyte changes in this type of shock resemble those in adrenal insufficiency qualitatively, they differ so greatly quantitatively that there is little reason at present to assume a similarity of causes.

METHOD. The condition of shock was produced in dogs (5–9 kgm.), under light ether anesthesia, by inflicting 1000 to 1500 light blows with a rubber mallet on a small fleshy area near the posterior margin of each thigh. This required ½ to ¾ of an hour and the tissue in the region of injury usually felt soft and spongy at its termination. Dissection at death revealed that the muscle was not pulped as had occurred in the earlier experiments of Best and Solandt but that the

[1] The data reported here were presented at the meeting of the American Physiological Society, Chicago, 1941 (This Journal **113**: P376, 1941).

spongy texture was due to fluid accumulation in the subcutaneous and intermuscular fat and connective tissue. On rare occasions the fibers of a muscle bundle directly below the pounded area were found to be severed transversely, but elsewhere in the same region the muscles were intact although usually red and swollen in appearance. The animals recovered from anesthesia and were bright and active for about 3 to 17 hours (average = 8.7 hr.) when they succumbed in secondary shock. The condition was manifested by weakness, a fall in blood pressure and final prostration; the blood pressure was measured as rapidly as possible before death by connecting a cannula in the carotid artery to a mercury manometer.

Blood samples were taken from the external jugular vein before anesthesia and at varying intervals following the muscle trauma. Jugular vein plasma was chosen to represent blood coming *from* a tissue and hence least likely to exhibit potassium changes since the tissue might be expected to absorb some of the incoming potassium. As the blood pressure fell in shock it was necessary to procure the terminal sample from the carotid artery and the heart. The heparinized blood was centrifuged at once, the hematocrit values noted and the plasma removed from the cells. Chloride was determined on 0.3 to 0.5 ml. of plasma. Samples of 0.5 to 1.0 ml. of plasma were weighed in platinum crucibles and dried in an oven overnight at 100°C in order to estimate the water content. A little sulfuric acid was then added to the crucibles and the plasma samples ashed in a muffle furnace overnight at about 550°C. It was found that the Shohl and Bennett potassium method as described by Fenn et al. (6), when modified slightly, could be used satisfactorily on such small samples. The average difference between 39 duplicate samples was 2.8 per cent. Chloride was determined by the Van Slyke procedure as used by Manery, Danielson and Hastings (8) and sodium by the Butler and Tuthill technique (4).

RESULTS. 1. *Potassium.* (a) *Plasma K in control dogs.* The concentration of potassium in dog plasma drawn in the mornings (table 1) from the external jugular vein ranged from 12.2 to 20.0 mgm. per 100 ml. in 44 normal dogs with an average value of 15.9 mgm. (p.e. ± 1.7 mgm.). This range of individual variation from one dog to another is somewhat less than others previously reported (2, 9). To estimate the extent of the variation in any one animal, two pre-anesthetic samples were taken 20 to 35 minutes apart, from each of 14 dogs. The average difference between the two samples was 5.4 per cent (p.e. = ± 3.2 per cent). In 8 cases the second sample showed a lower potassium value than the first (see dogs 18, 24 and 26, fig. 2), which may be due to the adrenalin secreted as a result of the first venous puncture. A single injection of adrenalin has been shown to cause an immediate and transitory rise in plasma potassium followed by a secondary and somewhat more prolonged fall (7). In the cases in which blood was drawn from the same animal on several successive days the plasma potassium showed remarkable constancy from day to day.

Control experiments were performed by exposing dogs to ether anesthesia for ½ to 1 hour, allowing them to recover and withdrawing blood at intervals throughout the day. The complete data from 4 representative cases are

TABLE 1

Plasma potassium concentrations and changes in hematocrit values (ΔC.V.) (in per cent of the initial value) following muscular trauma

DOG NO.	DOG WT.	SAMPLE 1		SAMPLE 2			SAMPLE 3			SAMPLE 4			SURVIVAL TIME
		K	C.V.	Time	K	ΔC.V.	Time	K	ΔC.V.	Time	K	ΔC.V.	
Group A—Died. Terminal sample obtained													
	kgm.	*mgm. per cent*	*per cent*	*hr.; m.*	*mgm. per cent*	*per cent*	*hr.; m.*	*mgm. per cent*	*per cent*	*hr.; m.*	*mgm. per cent*	*per cent*	*hr.; m.*
5	5.7	19.6	51				*2; 30	44.5	−20	†2; 40	47.3	−20	2; 40
6	8.6	15.9	52	3; 45	15.8	+10	*5; 25	33.4	+10	†5; 35	42.0	+13	5; 35
7	6.4	17.0		1; 5	14.8		5; 0	17.0		†6; 25	44.0		5; 50
8	7.7			4; 15	14.7					6; 15	26.9		6; 17
4	9.1	14.1		4; 25	17.3		5; 25	17.5		6; 31	25.7		6; 36
9	7.3	16.8	60	5; 20	17.3	−25	*7; 10	21.1	−19	†7; 30	30.6	−18	7; 30
10	7.7			1; 39	16.1		5; 40	18.5		7; 25	19.6		8; 35
11	5.7	15.3	38	5; 20	15.2	−16	8; 10	25.0	−11	†8; 35	33.8	−19	8; 35
12	6.1	17.5	58	6; 35	19.0	−10	*10; 5	21.9	−11	†10; 14	23.0	−11	10; 14
13	5.0	16.9	34	6; 5	22.8	−28				16; 50	20.5		17; 5
14	4.5	14.6	50	7; 45	24.6	−5	*17; 0	23.3	−2	†17; 35	46.4	+2	17; 35
Group B—Died. No terminal sample obtained													
15	12.7	18.4	47	1; 35	13.0	−11	3; 50	15.8	−11				5; 35
16	7.0	15.6	48				4; 30	15.3	−8				5; 45
17	7.7	18.5	48	1; 55	20.6	+4							7–9;
18	8.6	16.3		2; 40	16.9		4; 48	19.9					8; 40
19	5.2	17.0	50	2; 40	21.3	−7							10; 50
20	5.4			7; 15	22.1								8–10;
21	7.3	15.5		0; 45	20.4		7; 35	27.4					8–16;
Group C—Lived													
22	7.5	14.5		1; 15	16.6		5; 15	14.5		8; 10	14.3		Lived
23	6.8	16.1	39	1; 35	15.6	+4	5; 20	15.9	−9				Lived
24	8.2	16.4		3; 0	19.1		7; 40	19.6		10; 35	18.3		Lived
25	6.8	12.8		1; 7	15.0		8; 5	16.6					Lived
26	7.3	16.6	58	4; 50	17.3	+3	10; 20	17.6	+5	14; 0	20.2	−10	Lived
Group D—Cord cut, C; sympathectomy, S													
1 C	5.9	13.6					1; 0	20.4		*1; 11	28.4		1; 14
2 C	5.4			4; 17	17.9		7; 15	19.4		10; 45	18.1		15–17;
3 C	5.7	16.6	45	3; 20	20.2	−29	5; 19	18.6	−31	8; 55	18.9	−32	Lived
1 S		16.1		4; 53	15.1		6; 35	16.2		8; 35	15.4		Lived

All times refer to the intervals which elapsed after the commencement of trauma.

Sample 1 is the initial pre-anesthetic sample.

Sample 4 is a terminal sample in those dogs which died. All plasmas were drawn from the jugular vein except those with an asterisk which were from the carotid artery and those marked with a dagger which were cardiac samples.

The dogs in group D were either sympathectomized or had the spinal cord transected 2-6 days before the experiment.

plotted in figure 1. In order to facilitate comparison of the control values with those of the traumatized dogs it was found convenient to divide the total period of observation into the following intervals, the first 40 per cent, the second 40 per cent, and the final 20 per cent. Ether (2) and other anesthetics (7) have previously been reported to lower plasma potassium. Although no effort was made to record this fall, it is evident in some of the individual curves (fig. 1) and is sufficient to produce an average decrease of 8 per cent of the pre-anesthetic concentration during the first 40 per cent of the period studied. During the next time interval of 40 per cent the concentration returned to normal (average

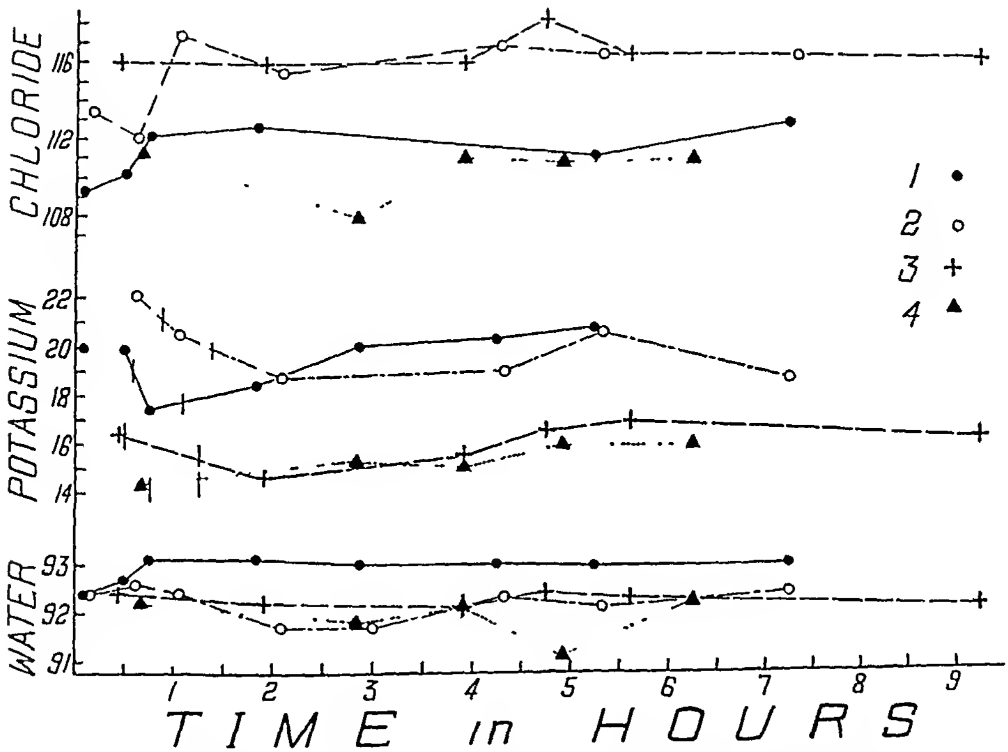

Fig. 1. The chloride (in m. eq. per l.), potassium (in mgm per 100 grams) and water (in grams per 100 grams) in the jugular vein plasma of 4 control dogs, which were subjected to ether anesthesia for the time intervals indicated by the distance between the two upright strokes on the potassium curves.

change, 0.6 per cent decrease of the initial value) thus showing the transitory nature of the effect of anesthesia. Although the average concentration did not rise above normal during this time, it is important to record that there was one potassium increase of 10 per cent, all others remaining below 6 per cent.

(b) *Plasma K increase due to muscular trauma.* The data obtained on 27 dogs are listed in tables 1 and 2 in the order of the periods of time survived following trauma. The first series included 13 dogs, 5 of which recovered (see group C, tables 1 and 2) while 8 succumbed. From these, blood samples were taken at 1 or 2 hour intervals during the experiment. Nine of the more complete time courses are plotted in figure 2. During the first 80 per cent of its

TABLE 2

Plasma water and chloride concentrations, magnitude of leg swelling at death and remarks concerning the condition of each dog

DOG NO.	SAMPLE 1		LATER SAMPLE			LEG SWELLING	REMARKS
	H_2O	Cl	Time	ΔH_2O	ΔCl		
			Group A. Died. Terminal sample obtained				
	gm. per cent	*m.eq./l.*	*hr.; m.*	*per cent*	*per cent*	*per cent Bl. vol.*	
5	92.0	117.0	*2; 30	+0.54	−7.4	32	Slight intraduodenal hemorrhage
6	92.6	107.5	3; 45	−0.43	−5.7	71	Note hemoconcentration and leg swelling
7	92.5	102.6	5; 0	+0.75	−3.2		Vomiting. Dog in shock at 5 hours
8			6; 15	+0.32	−2.7	47	Some spreading of blood through abdominal wall
4	92.0	114.5	5; 25	−0.54	−2.0		Shock in 6 hr., B.P. = 80. In 6 hr. 26 m., B.P. = 36
9	92.4		*7; 10	+0.22	−11.4	48	Extensive spreading throughout abdominal wall
10	92.5	111.9	7; 25	−0.43	−10.2		In 5 hr. 50 m. in deep shock—B.P. = 57
11	93.0	119.5	8; 10	−0.96	−4.7	0	Slight intraduodenal hemorrhage
12	92.7	114.0	*10; 5	−0.21	−1.7	38	Marked intra-intestinal hemorrhage
13	92.6	107.9	6; 5	−1.4	−3.6	45+	Extensive spreading throughout abdominal wall
14	92.0		*17; 0	−1.3		45	In deep shock at 17 hours
			Group B. Died. No terminal sample obtained				
15	92.0	109.7	1; 35	+0.22	+2.0		Note that K was still low 2 hours before death
16	91.6		4; 30	+0.87		45	Marked hemorrhage in duodenum
17	92.4	107.3	1; 55	+1.1	+1.5	44	Did not recover from anesthetic
18	91.6	107.8	4; 48	−0.33	−6.8		
19	92.5		2; 40	0.0		48	
20			7; 15	+0.43	−8.4	46	B.P. seemed low at 7 hr. 15 m.
21	91.9	107.1	7; 35	−0.54	0.0		B.P. seemed low at 7 hr. 35 m.
			Group C. Lived				
22			8; 10		−2.1		Note that K rose, then fell
23	92.5	110.2	5; 20	+0.11	+4.3	0	No leg swelling in 7 hours
24		111.6	10; 35		−3.4		Note that K rose, then fell
25	92.0	120.6	8; 5	−0.54	−5.1		At 8 hr. 20 m. signs of physical weakness were evident
26	92.0	112.9	14; 0	−0.87	−10.0		Note that K rose although dog lived
Av.	92.2	111.5					

The interval of time listed under "later sample" is the period which had elapsed since commencement of trauma. ΔH_2O and ΔCl are expressed in per cent of the initial concentration except in a few cases when this value was not obtained; then the difference from an average value for H_2O of 92.2 per cent and for Cl of 111.5 was calculated. The asterisks refer to carotid samples; all others were from the jugular vein.

course, every curve shows a maximum rise in potassium concentration which is greater than 10 per cent of the pre-anesthetic value, this being the largest increase found in the controls. Of these maxima the smallest recorded in figure 2 was +22 per cent. It is of interest that these observations apply to dogs in which the trauma was not sufficiently severe to cause death, as well as to those which succumbed. Some of the dogs which lived may have suffered slight but not fatal shock, such as dog 25 (table 2), for example, which showed signs of weakness and prostration although it subsequently recovered. In any case a

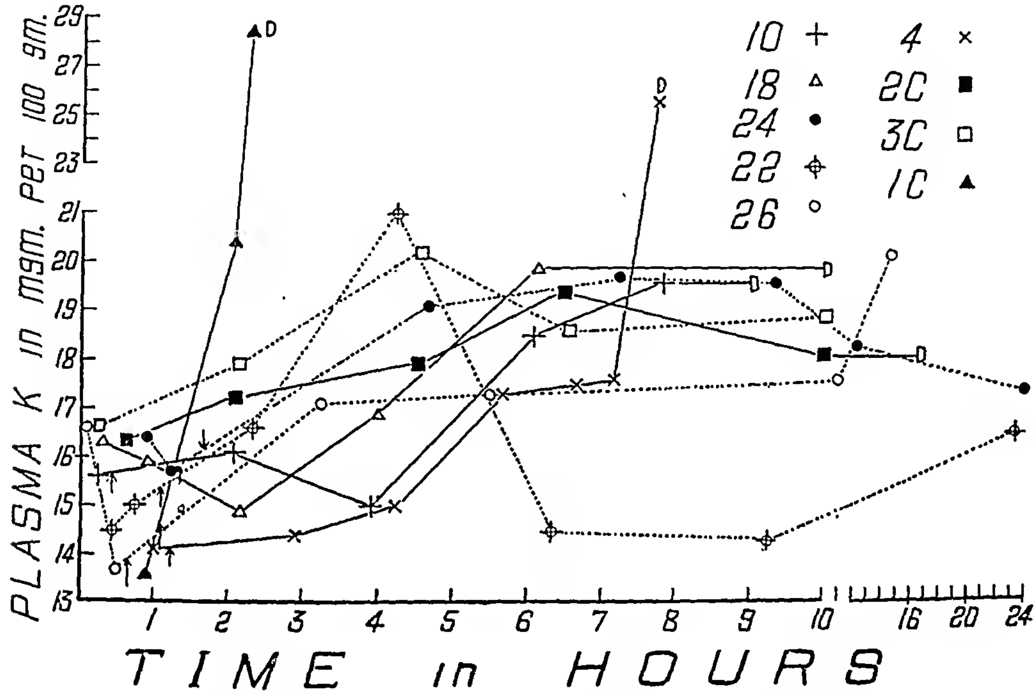

Fig. 2. The potassium concentrations in the jugular vein plasma of 9 dogs subjected to mild muscular trauma under ether anesthesia. When pre-anesthetic samples were obtained, arrows show the beginning of the traumatization which required ½ to ¾ of an hour. The 5 dogs marked with continuous lines died at the times indicated by *D*; the other 4 recovered. In dogs 2C, 3C and 1C the spinal cords were transected 2 to 6 days before the experiment.

comparison of these graphs (fig. 2) with those in figure 1 shows clearly that plasma potassium rises significantly as a consequence of muscular trauma.

In the next series considered all of the dogs died and only pre-anesthetic samples, samples taken several hours after trauma, and terminal samples were analysed. It is obvious from the character of the time courses plotted in figure 2 that this method of sampling will not always demonstrate the maximum increase in plasma potassium. Hence a considerable variation in sample 2, table 1, is to be expected. Nevertheless there were 9 cases in groups A and B where a significant rise was recorded in sample 2 and some of these were so large that an average increase of 13 per cent was obtained. Attention is drawn to group D in which it was shown that animals with spinal cords transected like-

wise exhibited an increase in the jugular vein potassium. In addition, two other dogs not listed were used for cross-circulation experiments after traumatization. The potassium increase of the traumatized donor was from 13 to 17 in one case and 15 to 29 in the other. Both uninjured recipients lived, one showing a plasma potassium change of from 15 to 12 mgm. and the other 14 to 17, the final values quoted being those found at the death of the donor.

In order to summarize the data a mass plot was made (fig. 3) of the observations on all of the dogs in the second series and on 3 of those in the first series

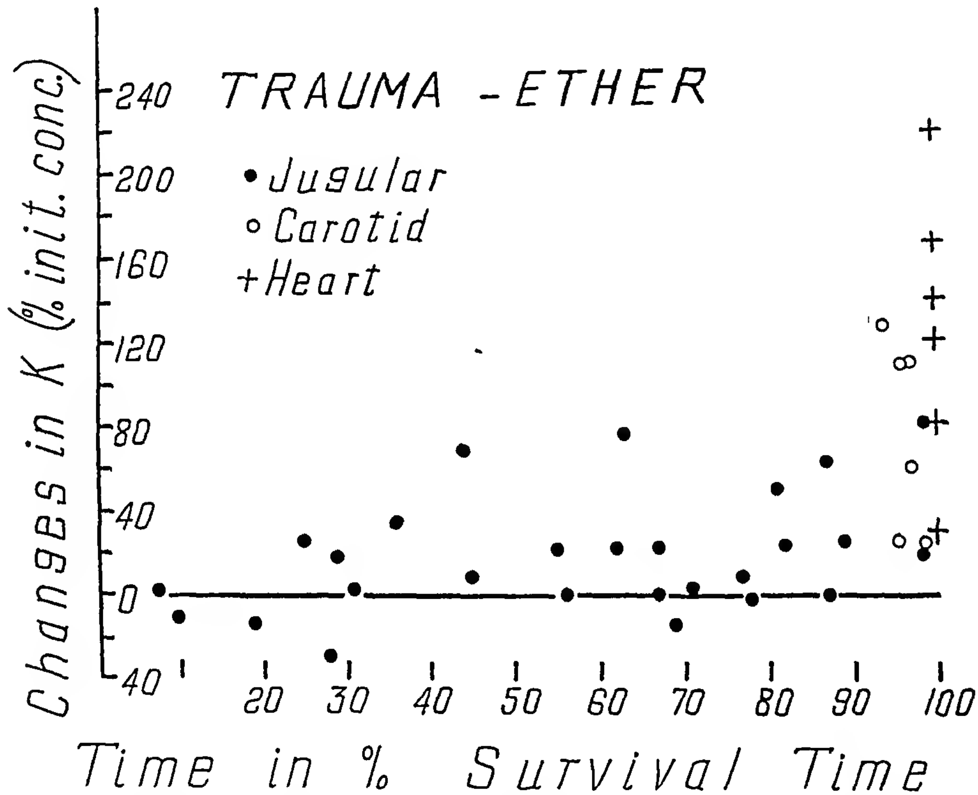

Fig. 3. A time course plot of the plasma potassium changes which occurred in 16 dogs between the commencement of muscular trauma and death due to secondary shock. Survival time is the period between the beginning of trauma and death, death being judged by the cessation of the heart beat.

which died, and for which pre-anesthetic values were obtained. The K-lowering effect of the anesthetic is evident early in the period of survival. However, the average over the first 40 per cent of the survival time was +4 per cent (p.e. ± 13 per cent) of the pre-anesthetic concentration. (Note the decrease of 8 per cent, p.e. ± 4 per cent, observed in the controls.) During the second time interval of 40 per cent the potassium rose sufficiently to give an average increase of 19 per cent (p.e. ± 18 per cent) which is considerably higher than the average decrease of 0.6 per cent (p.e. ± 4 per cent) observed in the controls and is twice the highest individual increase in the controls. Hence, although much

variation exists, the above figures illustrate that the chances are great that jugular vein plasma potassium will rise significantly as a result of trauma.

The potassium changes discussed above occurred considerably before the onset of death, but the greatest increase is evident in the last tenth of the period of survival. Although little significance is attached to this increase it cannot be attributed to the phenomenon of death alone because it was relatively small in some samples taken at death (see dogs 8, 4, 12 and 13, tables 1 and 2). The terminal samples are not strictly comparable with the earlier ones since they were taken from the carotid artery or from the heart. The reason for the increase at death is not definitely known. Dennis and Moore (5) report a large loss of potassium from the heart into the coronary veins after 5 to 9 minutes of ischemia produced by ligating the coronary arteries. Attention is drawn to dog 7 from which a heart blood sample was procured 35 minutes after death. If the terminal rise in potassium in heart plasma were due to its liberation from heart muscle because of anoxia of the tissue a much higher value than 44 mgm. per 100 ml. might be expected, since the potassium concentration in normal heart tissue is close to 400 mgm. per 100 ml. of tissue water. However, if either the liver or the heart liberated a certain fraction of its potassium (10) at some point in the progression of asphyxial symptoms at death, this would account for the fact that a maximum value seems to be reached which is not surpassed even in blood exposed to heart muscle for ½ hour after death (dog 3).

(c) *Tissue analyses.* No matter what is the source of the terminal rise in potassium, it is reasonable to suspect that the increase earlier in the period of survival is due to liberation from the injured muscle. Some preliminary analyses of tissues and of fluid collected from the region of injury (trauma fluid) are assembled in table 3. Although no large blood vessels were severed as a result of the traumatization, dark red fluid gathered in the subcutaneous tissue. This fluid could be collected and measured. After centrifuging, the supernatant showed a lower water content, a lower chloride concentration and a much higher potassium concentration than plasma. The results suggested that plasma and some cells leaked into the area and that to the resulting fluid had been added potassium from injured muscle cells and muscle cell solids. It should be recalled that, since dog red cells have a preponderance of sodium rather than potassium, hemolysed cells will not contribute appreciably to the potassium of the fluid. In a few cases femoral arterio-venous potassium differences were determined and found to be surprisingly small. Although this indicates slow leakage of potassium into the general circulation through venous channels, it does not preclude rapid entrance via lymphatics.

The tissues analysed give some evidence of the extent of the potassium loss. Using the triceps of the forelimb as a point of reference it can be seen (table 3) that the extensors of the knee are scarcely affected by the injury to a small portion of the flexors. The gastrocnemius shows some loss of potassium and gain in water and chloride. In dog 27 a muscle bundle was found to be broken as a result of the injury. Only the broken ends lost most of their normal potassium content. Tissue taken next to these ends still retained more than half

of its normal concentration. If 100 grams of muscle lost half of its potassium about 200 mgm. of potassium would be liberated, which seems a relatively large amount, although a normal animal could easily cope with it.

2. *Plasma water and chloride.* The changes in the water and chloride concentrations of the plasma were small and will not be presented in detail. Table 2 shows the normal pre-anesthetic values (sample 1) and the maximum percentage changes. Neither water nor chloride changed appreciably during the day in the control animals plotted in figure 1, but table 2 shows that following trauma there is a small but definite decrease in chloride concentration. In no case will the dilution of the plasma account for this fall. Only terminal changes are presented in the table but the fall in chloride concentration was gradual and

TABLE 3

Tissue and fluid analyses

(All figures are expressed in units per kilogram fresh tissue except the chloride of plasma and of trauma fluid which is in m. eq. per l.)

TISSUE	DOG 7			DOG 4			DOG 1C			DOG 27		
	H_2O	Cl	K	H_2O	Cl	K	H_2O	Cl	K	H_2O	Cl	K
	gm.	*m.eq.*	*m.eq.*	*gm.*	*m.eq.*	*m.eq.*	*gm.*	*m.eq.*	*m.eq.*	*gm.*	*m.eq.*	*m.eq.*
Triceps........	757	15.0	107.0	749	13.5	109.8	753	17.2	91.2	760	12.1	104.8
Extensors......		27.4	92.7	757	14.0	104.0	760	15.2	100.6			
Flexors.........		44.0								774*	63.5*	26.1*
Flexors.........										780†	41.9†	60.0†
Gastroc........				771	28.1	82.3						
Trauma fluid...				866	98.9	17.6					100.7	9.8
Plasma........				915	116.1	6.6	915		7.3	933	112.0	5.7

Muscles analysed were triceps of the fore limb which were far removed from the site of injury, extensors of the knee from the region of injury but not themselves damaged, and the flexors of the knee which were the injured muscles. Figures with an asterisk are analyses of the cut ends of a severed muscle bundle; while those with a dagger refer to the tissue adjacent to the cut ends. Trauma fluid is the supernatant obtained by centrifuging the fluid collected from the injured area.

progressive, the greatest always being found in heart plasma. These findings are not in accord with the results of Bisgard et al. (2) who report that the plasma chloride concentration is not altered in traumatic shock. The chloride is not decreased due to excretion since oliguria persists in these dogs. It could result in part from increased CO_2 tension, from the entrance of chloride-free intracellular water into the plasma or from the exchange of chloride for some anion in the injured area.

3. *Changes in red cell volume and local fluid loss.* The consistently small changes in red cell volume (table 1) and the constant water content of the plasma suggest that whole blood and not plasma or plasma water was lost at the site of injury. The hematocrit variations show more evidence of hemodilution than of hemoconcentration, although often a slight dilution was followed by subsequent concentration. That a considerable amount of whole blood is trapped in the

injured limbs is demonstrated by the extensive hemolysis evident in the trauma fluid; all jugular plasma samples taken after trauma likewise showed signs of hemolysis. The hemodilution which is evident in some cases may result from the entrance of extracellular or intracellular water from elsewhere in the body or merely from the retention of red cells in the injured area due to the clamping down of small vessels there. Hemodilution usually accompanies the type of shock produced by bleeding from a large artery but Blalock (3) obtained hemoconcentration as a result of slow and intermittent bleeding. Measurements of the volume of the injured limbs immediately after trauma and again at death (table 4) illustrate that considerable variation in the time of bleeding into the limbs can occur. In most cases the results suggest a slow bleeding throughout the course of the experiment. Hence, fairly constant hematocrit values are to be expected since several factors probably operate simultaneously, some tending to increase and others to decrease the red cell volume.

To estimate the swelling of the injured limbs each limb was immersed, up to a certain mark, in a container of water and the volume of water displaced before and after trauma and at death was measured. The method is in error if there is extensive spreading of the hemorrhage up the abdominal wall. This occurred in only 2 cases. In other instances when the spreading was slight the affected tissue was dissected and measured. In general, as table 4 indicates, the swelling is greater at death than just following trauma, suggesting that slow bleeding into the injured area typifies this type of shock.

To evaluate the contribution to the cause of death made by local fluid loss, the amount of limb swelling in these experiments was compared to the quantities of blood removed when death results from frank hemorrhage. In the latter case it is generally agreed that a loss of 50 per cent of the calculated blood volume is fatal. The swelling of the limbs is so great, particularly since the values obtained are minimal, that hemorrhage at the site of injury must be a major factor in the cause of death. In addition the blood volume will be further decreased by the blood taken for analysis and by that lost due to intra-intestinal hemorrhage (see table 4). However, although in many cases the amount of bleeding is itself sufficient to cause death, there are also instances in which the limb swelling is slight. There seems too to be little if any relation in group 1, table 4, between the survival time and the magnitude of the limb swelling. Furthermore, in the group of dogs which died the losses in all but 3 cases were less than 50 per cent of the calculated blood volume; and, of those which lived 24 hours or longer; 3 lost more than 50 per cent of the blood volume.

Discussion. The plasma electrolyte changes in shock have been likened by Scudder and his associates to the changes in adrenal insufficiency. The latter condition is characterized by decreased chloride and sodium concentrations accompanied by hemoconcentration, an increased solid content and an increased potassium concentration in plasma. In the condition reported in this paper there is no evidence of increased solid content in the plasma, and more evidence of hemodilution than hemoconcentration. The chloride changes (av. = 4.1 m.eq.per l.), although in the same direction as in adrenal insufficiency, are much

less than in most cases reported. We did not conduct a complete study of sodium changes but in the three dogs whose plasmas were analysed the concentration did not vary appreciably or consistently from the normal although the animals died of shock. The only real similarity to adrenal insufficiency is the

TABLE 4

Relation of local fluid loss to survival time

GROUP I—DIED				GROUP II—KILLED AFTER 24–30 HOURS		
Dog no.	Limb vol. increase (per cent bl. vol.)		Survival time	Dog no.	Limb vol. increase (per cent bl. vol.)	
	1	2			1	2
			hr.; min.			
1C		28	1; 13			
28	9.7	13	1; 20	39		22
5*	32	32	2; 40	40	16	
29†	32	43	3; 0	41	7	
30	35	52	3; 0	42	20	
31	33	46	5; 0	43	11	
6	47	71	5; 35	44	16	
16†	27	45	5; 45	45	12	
8		47	6; 17	46*	34	0
9	41	48	7; 30	47*	17	30
11*	24	0	8; 35	48	0	
17		44	7–9;	49	22	
20	37	46	8–10;	50	33	54
32		37	9 app.	51	33	42
12†	17	38	10; 14	52	17	47
19	13	48	10; 50	53	38	38+
33		33	11; 15	54*	14	51
34	43	63	11; 25	55	25	57
35*	24	42	11; 40	56	23	34
36		35	15; 20	23		0
37		34	15; 0			
13		45+	17; 5			
14*	17	45	17; 35			
38	15	38	24; 0			
Average ..	28	41			20	34

Limb volume increase expressed in per cent of a calculated blood volume (bl. vol. = 8 per cent of the body weight) was measured immediately after traumatization (1), and again at death (2). Dogs from 31 to 56 were carefully examined for intestinal hemorrhage. Those marked with a dagger showed severe intra-intestinal hemorrhage, while those with an asterisk showed a slight amount.

increased plasma potassium. Even this similarity is not particularly striking since a few animals succumbed without much change in the jugular vein potassium until within a few minutes of death. A precise comparison with adrenal insufficiency is difficult since the blood vessels used for sampling by each investigator differed, and also our dogs were exposed to the K-lowering effect of

anesthesia. Furthermore, because the largest increase occurred just at death, it is important to know the proximity to death of the samples taken in adrenal insufficiency. Muntwyler et al. (9) report values of 35 mgm. per cent (range 10–74) in femoral artery plasma of 11 dogs in "rather marked insufficiency." The average value simulates ours (table 1) although the range is much greater.

There is little to be said regarding the toxic effects of potassium in experiments where the fluid loss is so great that it could be the sole cause of death. Injection of potassium salts sufficient to raise the plasma concentration to 50–60 mgm. per cent was shown to cause death by intraventricular block and cardiac arrest (13). The shocked dogs did not die for this reason because even in heart samples at or after death (table 1) the concentration did not rise to such high levels. Little is known about the toxic effects of potassium other than those on the heart. Hence, although it is unlikely that potassium caused death, the concentration becomes abnormally high and it might indeed be a contributing factor.

The data presented here show clearly that a significant increase in jugular vein potassium follows muscular trauma, but there is some variation from dog to dog. This variability is to be expected if one accepts as a tentative working hypothesis the following sequence of events: that ether causes a transitory lowering of plasma potassium, that the injured muscle liberates potassium which is not excreted because of the oliguria which persists, that the tissues probably absorb as much potassium as they can under the conditions of the experiment.

SUMMARY

The blood of twenty-seven dogs was studied after the dogs had been subjected to mild trauma of the muscles under ether anesthesia. They succumbed in secondary shock in 3 to 17 hours after the commencement of the traumization. The condition of shock is characterized by considerable swelling in the injured regions, by a slight decrease or no change in the red cell concentration, and by little alteration in the chloride or water concentration of the jugular vein plasma. A small but significant increase in plasma potassium occurs considerably before death, and an increase of 100 to 200 per cent at or just prior to death. That local fluid loss was the major factor in the cause of death was concluded from a larger series of animals in which the swelling of each traumatized limb was measured.

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THE RÔLE OF OXYGEN IN THE METABOLISM AND MOTILITY OF HUMAN SPERMATOZOA

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The experiments reported here are a continuation of the work on human spermatozoa which was begun with the object of studying the metabolic behavior of these cells. In previous papers (7, 8), the metabolism was shown to be almost exclusively glycolytic, the oxygen consumption being of such small magnitude that it could not properly be interpreted as a true respiration. Furthermore, no spectroscopic evidence of cytochrome could be found and it was observed that, in certain cases, motility was profoundly depressed in the presence of pure oxygen. These observations have been confirmed in every essential respect by Ross et al. (12) except that their mean figures for glycolysis are higher than those given by this author (7). Since the latter results were published, higher and more consistent figures[2] have been obtained from the combined spermatozoa of a small new group of donors. These figures are in closer agreement with those of Ross et al. and show, furthermore, that the level of aerobic glycolysis is virtually the same as that of the anaerobic.

The present extension of these studies was undertaken to determine more precisely the behavior of the spermatozoa under aerobic conditions and particularly to examine the link between glycolysis and respiration in these cells. The experiments entailed 1, determination of the activity of certain dehydrogenase systems; 2, the measurement of oxygen consumption in the presence of different substrates, and 3, testing for the presence of the cytochrome complex by indirect methods. Lastly, the toxic effect of high oxygen tensions was investigated from the point of view of a possible formation of hydrogen peroxide by the spermatozoa.

Methods. The spermatozoa suspensions were prepared by methods described previously (7, 8). For the measurement of oxygen consumption, the cells were suspended in glucose-free Ringer-phosphate solution (pH 7.35) and shaken in Warburg manometers at 38°C. Various substrates were added directly to the cell suspensions in the Warburg vessels prior to temperature equilibration. The final concentration of each substrate was M/100. Cell motility was determined before and after every experiment by methods already

[1] Aided by a grant from the National Committee on Maternal Health.

[2] The new figures are as follows:

No. of expts.	60
$mm^3 CO_2/10^8$cells/hr. in N_2	25
$mm^3 CO_2/10^8$cells/hr. in O_2	24

It should be stated, however, that figures as high as these were obtained previously from some individual specimens (7).

described (8). For the determination of cytochrome and cytochrome oxidase activity, the p-phenylene diamine was added to the cell suspensions according to the techniques outlined by Keilin and Hartree (5) and Stotz et al. (13). These experiments will be described in detail below.

A modified Thunberg technique, which allowed simultaneous measurement of glycolysis and methylene blue reduction, was used to measure the dehydrogenase activity of the spermatozoa in the presence of certain substrates. The cell suspensions were prepared for the measurement of glycolysis (0.03M bicarbonate being substituted for phosphate) and equilibrated for 20 minutes at 38°C. with 95 per cent N_2—5 per cent CO_2. Methylene blue was placed in the side-arms of the Warburg vessels and added to the suspensions in the main vessels when equilibration was complete. Thereafter, at intervals, the manometers were removed temporarily from the water-bath to determine when reduction of the dye was complete.

Experiments. The dehydrogenases of the spermatozoa were investigated using a variety of substrates which included glucose, succinate, pyruvate, fumarate and lactate among others. Only in the presence of glucose and succinate was any rapid or marked reduction of the methylene blue seen.[3] The fastest reduction times were seen invariably in the presence of glucose and to a lesser degree when succinate was present. In the absence of substrate or in the presence of the other substrates mentioned above no marked reduction of the dye took place over a period of many hours. These results are in marked contrast to those of Lardy and Phillips (6) who showed that the reduction of methylene blue by bull spermatozoa took place rapidly in the *absence* of substrate and in the presence of succinate and fumarate but was inhibited when glucose was added.

The importance of the above results for human spermatozoa lies not so much in the reduction time values but in the active reduction in the presence of succinate. This was the first positive evidence obtained of the presence in the cells of the succinic dehydrogenase and, therefore, of the probable presence of the cytochrome system (2). This evidence was amplified further when malonate (M/100), a known inhibitor of the succinic dehydrogenase (11), was added to the spermatozoa suspensions reducing the dye in the presence of succinate. Malonate almost completely inhibits this reduction but has no effect on the reduction time in the presence of glucose.

Effect of succinate on the oxygen consumption. These results suggested a re-examination of the oxygen consumption of the spermatozoa particularly in relation to the presence of the cytochrome complex. It seems definitely established (2) that the succinic dehydrogenase is, structurally and chemically, intimately linked with the cytochrome system. When the dehydrogenase is reduced, it is oxidized by cytochrome C almost instantaneously (4) and not to any appreciable

[3] There are certain objections to the use of intact cells for the measurement of enzyme activity, mainly that of the possible difference in penetration of the intact cell of different substrates. The figures given in table 1 serve merely to indicate that enzymatic activity towards a particular substrate is present and are not intended to demonstrate the *maximal* activity toward that substrate.

extent by molecular oxygen. As shown above, methylene blue can also act as the hydrogen acceptor. Therefore, it seemed probable that, if succinate was substituted as a substrate for glucose, the oxygen consumption of the spermatozoa would be increased. This proved to be the case. The figures in table 1 show the results obtained when oxygen consumption was measured in the absence of substrate and in the presence of glucose and succinate.

The figures obtained in the presence of glucose are similar to those reported previously (7). The increase in oxygen consumption in the absence of substrate, while small, is significant since it appeared in every experiment. The inhibiting effect of glucose has also been noted in ejaculated bull spermatozoa (6) but not in cells obtained from the epididymis (3). However, in the presence of succinate the human cells show a relatively high and stable oxygen consumption (fig. 1). In every case it was evident that the oxidation of succinate was vigorous and progressive compared to that of glucose. The addition of malonate or of azide inhibits the succinate oxidation to the autoxidation level. This evidence supplements that given above for the inhibition of methylene blue reduction by malonate in the presence of succinate. It also complements the observations of other authors (2, 4) which show the oxidation of succinate to be mediated by the azide-sensitive cytochrome complex.

TABLE 1

Oxygen consumption of human spermatozoa in the presence of different substrates

NO. OF EXPTS.	NO. SUBSTRATE	GLUCOSE	SUCCINATE	p-PHENYLENE DIAMINE (M/50)
20	1.43*	1.34	6.3	18

* The figures represent the oxygen consumption per $mm^3/10^8$cells/hour.

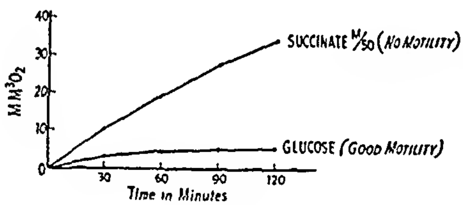

Fig. 1

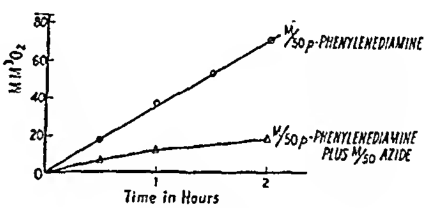

Fig. 2

Fig. 1. The oxygen consumption and motility of human spermatozoa in the presence of glucose and sodium succinate.

Fig. 2. The oxidation of p-phenylenediamine by human spermatozoa.

Neither fumarate, lactate or pyruvate increases the oxygen consumption above the autoxidation level. The failure of the spermatozoa to oxidize lactate is to be expected since they produce as much lactic acid in oxygen as they do in nitrogen. Their failure to oxidize fumarate, on the other hand, suggests that the oxidation of succinate is a one-step process, namely, the removal of hydrogen to form fumaric acid and the subsequent accumulation of the latter compound.

In spite of the vigorous oxidation of succinate by the spermatozoa, any energy made available in this reaction is not coupled with motile activity. In *all* experiments in which succinate was substituted for glucose, the motility failed as rapidly as if no substrate was present.

The oxidation of p-phenylenediamine. As a further check on the presence of the cytochromes and cytochrome oxidase in the spermatozoa, the ability of the cells to oxidize p-phenylenediamine was determined. Preliminary experiments showed that the autoxidation of this substance was negligible but that it was rapidly oxidized by the spermatozoa. The concentration necessary to produce maximal oxidation was M/50. The results are shown in table 1.

Keilin and Hartree (5) and Stotz et al. (13) have shown that the oxidation of p-phenylenediamine is mediated through cytochrome C and cytochrome oxidase and that its oxidation can be used as an indicator of the presence of such a system in cells. These authors have shown further that cytochrome B can be oxidized by molecular oxygen, but is insensitive to the inhibitory effect of cyanide and azide, and therefore can act independently of cytochrome oxidase. Stotz et al. (13) demonstrated a relatively cyanide-insensitive oxidation of p-phenylenediamine and showed conclusively that the remaining catalysis was due to cytochrome B. In the case of human spermatozoa approximately 20 per cent of the oxidation of p-phenylenediamine escapes azide inhibition (fig. 2). In line with the evidence of Stotz et al., this can be attributed to the presence of cytochrome B.

In view of the evidence given above, it is reasonable to conclude that in spite of the very small oxygen consumption of human spermatozoa, these cells contain a virtually complete respiratory system, namely, succinic dehydrogenase, cytochromes B and C and cytochrome oxidase,[4] and are capable of carrying on oxidative processes if the appropriate substrate is present.

The effect of high oxygen tensions on motility. Any analysis of the rôle of oxygen in the metabolism and motility of human spermatozoa would not be complete without a consideration of the phenomenon first described by this author (7) and confirmed by Ross et al. (12), namely, that of the loss of motility which often occurs when these cells are exposed to oxygen at 38°C. for several hours (table 2). It has already been shown that the spermatozoa will retain maximal motility in nitrogen at 38°C. for many hours (7). This evidence, coupled with the low oxygen consumption of the cells and their ability to get all their motile energy from a process which involved the breakdown of glucose to lactic acid, suggested that the toxicity of oxygen may be related to a similar process which retards the growth of certain anaerobic bacteria in air. MacLeod and Gordon (9) have shown that certain of these bacteria under aerobic conditions produce

[4] The absolute amount of these substances in the spermatozoa is exceedingly difficult to determine. Only a few milligrams of tissue (wet) are available for any given experiment and, since isolation of enzymes requires relatively large amounts of fresh material, certain extensions of this work on human spermatozoa are severely limited. As would be expected, the addition of cytochrome C to the intact cells in the presence of p-phenylenediamine causes no increment in the oxygen consumption.

enough hydrogen peroxide in the course of their metabolism either to destroy the growing colony or prevent its multiplication. Accordingly, experiments were designed to determine whether a similar phenomenon in human spermatozoa might be responsible for the loss of motility in high oxygen tensions. Two spermatozoa suspensions were set up in the usual way for measurement of aerobic glycolysis and equilibrated with 95 per cent O_2—5 per cent CO_2 for 15 minutes at 38°C. In similar fashion, another two suspensions of the same spermatozoa were set up, except that 0.1 cc. of dilute hemoglobin or catalase[5] was added to each on the assumption that if any hydrogen peroxide was produced in the system, it would be destroyed instantaneously either by the peroxidase activity of the hemoglobin or the catalatic activity of the catalase. The manometers containing the spermatozoa exposed to oxygen were then run at 38°C. for periods up to 9 hours and the motility examined at the end of the experiment. Typical experiments are shown in table 2. In every case where loss of motility occurred in the presence of oxygen, the addition of hemoglobin or of catalase was sufficient to maintain a maximal motility comparable to that of the same spermatozoa under anaerobic conditions.

TABLE 2

Spontaneous loss of motility in 95 per cent oxygen and the protective effect of catalase

EXPT. TIME	ACTIVITY OF SPERMATOZOA AT END OF EXPERIMENT		
	95% nitrogen—5% CO_2	95% O_2—5% CO_2	95% O_2—5% CO_2 and Catalase
hours			
8	4 (58%)	No motility	4 (55%)
8	3+ (50%)	No motility	3+ (52%)
7	4 (60%)	No motility	4 (50%)
9	3 (50%)	No motility	3 (49%)
5	3+ (32%)	1 (10%)	3+ (32%)
5	4 (68%)	2 (10%)	4 (70%)

The figures in parentheses denote the number of motile cells. The other figures denote the quality of motility, 4 being maximal motility.

These results were indicative of the production of hydrogen peroxide by the spermatozoa. It followed, therefore, that its formation and the resulting toxicity must be within the limits of the small oxygen consumption of the spermatozoa. This assumption was tested by adding amounts of hydrogen peroxide with varying oxygen equivalents to spermatozoa at 38°C. and determining its effect on motility. It was found that a concentration of peroxide[6] equivalent to between 10 and 20 mm.^{3} of oxygen was sufficient to destroy the motility of 200 million spermatozoa within 5 minutes. These oxygen equivalents are within

[5] I am indebted to Dr. Kurt G. Stern of Yale University for a generous sample of catalase of known purity.

[6] The oxygen equivalent of peroxide was determined by adding catalase to measured amounts of peroxide at 38°C. and measuring manometrically the oxygen produced.

the range of oxygen consumption which might be expected of the spermatozoa over a period of several hours. These experiments serve to demonstrate the sensitivity of the spermatozoa towards low concentrations of peroxide and indicate the nature of the chemical mechanism for the loss of motility which occurs when the cells are exposed to high oxygen tensions. Lardy and Phillips (6) have observed that bull spermatozoa show a similar sensitivity towards hydrogen peroxide.

Further investigation of the effect of oxygen on spermatozoa motility is in progress and preliminary results show that at low oxygen tensions (between 5 and 10 per cent) the toxicity is eliminated.

Discussion. The results presented here and those published previously (7, 8) indicate that oxygen is not of primary importance in the metabolism of human spermatozoa and is not essential for the maintenance of motility. On the other hand, the presence in these cells of a more or less complete respiratory system would suggest 1, that they once possessed a high respiratory activity, or 2, that their ultimate function required oxidative activity. Recent work on the metabolism of bovine epididymal spermatozoa (3) shows that the sperm in the epididymis rapidly oxidize glucose but, when ejaculated, they not only fail to oxidize glucose but the respiration is actually inhibited by this substance (3, 6). It is possible that a similar metabolic change takes place in human spermatozoa in passage from the epididymis to the seminal fluid.

In view of the rate of p-phenylenediamine oxidation, the cytochrome system is potentially capable of carrying a large oxygen consumption. That it does not do so, except in the oxidation of succinate, cannot be explained at present although it is obvious that the link between glycolysis and respiration has been broken or perhaps never existed. The spermatozoa cannot oxidize lactate or pyruvate and there is no evidence of the Pasteur effect. In the latter respect, the metabolism of human spermatozoa is, like that of jejunal mucosa (11), unique among mammalian tissues.

The conclusion has already been made (8) that human sperm derive enough energy for motility from the breakdown of glucose to lactic acid. This conclusion receives additional support from the evidence given here that the oxidation of succinate, presumably an energy-yielding reaction, does not maintain motility in the absence of glucose.

The failure of motility which often appears at 38°C. in high oxygen tensions and which can be prevented by catalase (or peroxidase) points definitely to the production of hydrogen peroxide in the system. The evidence is only indirect since the peroxide cannot be detected chemically. But the small amount of peroxide necessary to destroy motility is not easily susceptible of chemical analysis if, indeed, it exists as such for any length of time. That peroxide should be produced at all in the course of the metabolism of the spermatozoa is a matter of considerable interest since no formation of this substance has as yet been demonstrated in the cells of higher organisms (10). But such cells invariably have an active respiration and contain enough catalase rapidly to destroy any peroxide which may be formed (10). The virtual absence of respiration in the

spermatozoa and their pronounced sensitivity towards peroxide poisoning suggests a deficiency of catalase in these cells.

Finding the source of the peroxide in the metabolism of the spermatozoa awaits further analysis of the enzyme systems. Autoxidation of a flavoprotein is a strong possibility since hydrogen peroxide is the end-product of such a reaction. Theorell (14, 15) has shown that the reoxidation of flavoprotein is normally accomplished by the terminal respiratory system (cytochrome C) but if it can-not do so, it will react directly with molecular oxygen, particularly at *high oxygen pressures.* Since the cytochrome system in the spermatozoa is so inactive and since the possible peroxide formation takes place only at high oxygen pressure, the mechanism suggested above is tenable.

SUMMARY

1. The rôle of oxygen is not of primary importance in the metabolism and motility of human spermatozoa.

2. These cells possess a complete terminal respiratory system but can not oxidize glucose or its anaerobic breakdown products, lactate and pyruvate.

3. Succinic acid is oxidized but this reaction is not coupled with motility in the sense that any energy made available can be used for motile activity.

4. In regard to the depressing effect of high oxygen pressure on the motility, evidence is presented suggesting the production of small amounts of hydrogen peroxide by the spermatozoa and a possible mechanism for its production is discussed.

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OBSERVATION ON THE VARIOUS FACTORS INFLUENCING THE INCREASE OF ERYTHROCYTIC FRAGILITY INDUCED BY STASIS[1]

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That erythrocytic fragility is increased by stasis of the blood *in vivo* and *in vitro* has been demonstrated by Waller (1939), Tsai, Lee and Wu (1940) and Ham and Castle (1940). Tsai, Lee and Wu have shown that this phenomenon is not related to pH change nor to the lowering of O_2 tension and increase of CO_2 partial pressure in the stagnant blood. In a personal communication Prof. W. B. Castle has expressed his view that the increase of fragility is not due to an effect on the membrane, but rather to an increase of osmotically active material within the red cell. The present investigation is an attempt to test the above suggestion and to study various other factors responsible for the increased fragility induced by artificial stasis.

Methods. Dog's blood was used in all experiments. It was obtained aseptically by venipuncture. The blood after being withdrawn was immediately divided into 4 to 6 samples. One sample was used at once for fragility and lactic acid determinations, while the rest were kept in a water bath at 37°C. for varying periods from 1 to 20 hours before the tests. In prolonged experiments all the blood containers were sterilized beforehand.

The erythrocytic fragility toward hypotonic buffered saline was determined according to the method of Creed as modified by Tsai, Lee and Wu (1940). The degree of fragility was expressed in terms of percentage concentration of sodium chloride in the buffered solution that caused 50 per cent hemolysis. This was called mean corpuscular fragility (M.C.F.) by Creed (1938) and this term will be adopted in the present paper. It was obtained graphically by interpolating the percentage values of hemolysis.

The lactic acid content of the whole blood, of the cells and of the plasma was estimated by the method of Friedeman, Shaffer and Cotonio as described by Peters and Van Slyke (1931). In some experiments the blood cells were washed thrice with isotonic phosphate buffered saline (pH 7.25) and then made up with the same fluid to the original volume of the blood. They were subjected to artificial stasis as the whole blood. Since it was found that the washed cells did not exhibit increasing fragility with stasis, the lactic acid content of the cell suspensions with and without previous addition of glucose was also determined.

In another series of experiments the blood was made hypertonic by adding

[1] Preliminary reports of the present work have been published in abstract form in Proceedings of Chinese Physiological Society, Chengtu Branch, October, 1941 and February, 1942.

excessive glucose or citrate before starting artificial stasis. As this was shown to prevent the increase of fragility of the stagnant blood *in vitro,* several experiments were performed in which the stasis of blood was produced within certain organs. In two experiments with luminalized dogs we used two kidneys and two hind limbs as the stagnant organs; one side served as control, while the other side was injected with 5 to 9 ml. of 5 to 10 per cent sodium citrate or 5 to 8 ml. of 10 per cent glucose solution. In another experiment the spleen was employed in place of the legs. The viscus was tied into two approximately equal halves. In all these three experiments the organs were occluded by first ligaturing all the veins and next the arteries. Immediately after the occlusion of all the vessels a control blood sample was removed from the organ for fragility test. This was followed by an intra-arterial injection of glucose and citrate respectively into one kidney or one limb or a separated half of the spleen. Samples of the stagnant blood from these organs with and without previous glucose or citrate injection were removed after varying intervals of 2 to 5 hours and estimated for osmotic resistance as usual. Care was taken to check the colorimetric value of each sample against a common standard and due corrections were made for the percentage reading of hemolysis accordingly. Control tests regarding the possible influence of the hypertonic blood on the tonicity of the testing buffered saline were also performed and showed that the amount of the hypertonic blood used (0.02 ml.) was too small to produce noticeable alteration of the tonicity of the saline (2 ml.).

RESULTS. *Relation to lactic acid content of the blood.* When the blood was kept *in vitro* at body temperature for varying periods of time, erythrocytic fragility increased with lactic acid concentration of the whole blood. Table 1 records the results of a typical experiment for illustration. Although this increase is not exactly parallel, our calculation based upon 15 experiments has shown that the correlation coefficient of these two variables reaches nearly 0.5. However, this may be a coincidence and does not give a convincing proof of their causative relation.

If the increase of lactic acid in the stagnant blood is the cause of increasing fragility, we should expect to find no alteration of corpuscular resistance by preventing the formation of lactic acid with sodium fluoride, and a more marked increase by previous addition of lactic acid to the blood. Regarding the first point, our experiments were unsuccessful because sodium fluoride itself lowered the osmotic resistance of the red cells. The evidence regarding the second point is also equivocal because the increase was much smaller than it should be if lactic acid accumulation were the sole cause of increased fragility. The results from a typical experiment given in table 2 suffice to clarify our contention.

The effect of washing. In the washed cells the increase of fragility by artificial stasis was not observed. This is illustrated by the data from a typical experiment given in table 3. It was usually observed that after a short time of incubation the fragility drops to a lower level and tends to rise only very slightly during the next two hours; it remains slightly below the original level even after 5 to 10 hours.

This remarkable phenomenon was not altered by adding glucose to the cell suspension (table 4), or replacing the buffered saline with old plasma standing at room temperature over 10 hours (table 5). On the other hand, once the fragility has been rendered high by prolonged stasis of the whole blood, subsequent washing of the cells cannot prevent the further fall of osmotic resistance by re-subjecting to incubation (table 5).

TABLE 1

Relation of increased fragility to lactic acid concentration

	TIME (MINUTES)				
	0	105	165	420	545
M.C.F., NaCl gram per cent............	0.273	0.281	0.288	0.315	0.324
Lactic acid mgm. per cent.	4.93	5.30	21.6	33.1	36.9

TABLE 2

The effect of adding lactic acid to the blood before subjecting to stasis

	DURATION OF STASIS (HOURS)				
	Immediately	4.5 hours later			
	Lactic acid added (mgm. per cent)				
	0	0	20	30	40
M.C.F., NaCl gram per cent............	0.258	0.276	0.278	0.284	0.287
Lactic acid found mgm. per cent........	17.0	20.8	43.0	45.0	50.6

TABLE 3

Influence of washing on fragility

DURATION OF STASIS	M.C.F. IN NaCl GRAM/100 ML.	
	Whole blood	Washed blood
minutes		
0	0.335	0.338
90	0.337	0.320
240	0.348	0.326
300	0.354	0.328

Lactic acid formation in the washed cells. From the data presented in the preceding sections it appears that some substance responsible for the increased fragility induced by stasis may have been removed by washing. In order to ascertain whether glycolysis stopped after washing, we have carried out some experiments in which the formation of lactic acid in the unwashed and washed cells was determined. As shown in table 6, lactic acid formation practically ceases after washing. However, glycolysis is not a prerequisite for the increased fragility because by adding glucose to the cell suspension the formation of lactic

acid resumes without being accompanied by the lowering of osmotic resistance (see table 4).

The effect of adding or injecting glucose and citrate. If the increased fragility induced by stasis is due to the accumulation of osmotically active substance within the cell which causes a higher osmotic pressure in the cell interior than its

TABLE 4

The effect of adding glucose to the cell suspension on the M.C.F.
(results of two typical experiments)

DURATION OF STASIS	CONTROL	1 MGM. GLUCOSE ADDED TO 1 ML.		CONTROL	2 MGM. GLUCOSE ADDED TO 1 ML.	
	M.C.F.	M.C.F.	Lactic acid mgm./100 ml.	M.C.F.	M.C.F.	Lactic acid mgm./100 ml.
hours						
0	0.329	0.326	9.0	0.313	0.315	7.2
3	0.332	0.332	16.7	0.306	0.308	17.8
6	0.332	0.336	22.5	0.307	0.303	34.4

TABLE 5

The influence of old plasma on the fragility of the washed cells (results of a typical experiment)

DURATION OF STASIS	M.C.F. IN NaCl GRAM/100 ML.	
	Fresh cell + old plasma	Old cell + old plasma
hours		
0	0.304	0.319
4.5	0.300	0.318
5.5	0.301	0.338
6.5	0.306	0.343

TABLE 6

Lactic acid formation in the washed cells

UNWASHED CELLS		WASHED CELLS	
Duration of stasis	Lactic acid mgm./100 ml.	Duration of stasis	Lactic acid mgm./100 ml.
hours		*hours*	
0	12.8	2.5	8.23
2	20.6	4.5	9.02
5	24.7	6.5	9.76
7	28.8	23.0	9.25

environment, an artificial increase of osmotic pressure in the latter will balance that in the former and hence prevent the unequal migration of water molecules into the cell. With this as a working hypothesis, a number of experiments have been performed in which the cell environment was made hypertonic by adding glucose and citrate to the blood before subjecting to stasis. If the increase of osmotic pressure in the cell interior be the cause of increased fragility,

addition of slowly diffusible substance such as glucose or citrate would protect the cell.

The results from both *in vitro* and *in vivo* experiments in this series are concordant in demonstrating the fact that the increased fragility can be prevented by adding or injecting glucose or citrate to the blood before stasis. For this effect to manifest itself clearly, the glucose or citrate concentration in the blood must be high. In our *in vitro* experiments we have found that 0.5 per cent glucose or citrate in the blood produces only a very feeble effect, or in some cases no effect at all, but marked effect was observed in all cases when the final concentration reaches 2.0 per cent. In the *in vivo* experiments we have not determined the final concentration of glucose and citrate in the stagnant blood. In tables 7 and 8 are given some typical results.

TABLE 7

Influence of adding glucose and citrate to the oxalate blood before stasis

	M.C.F. IN NaCl GRAM/100 ML.		
	Duration of stasis (minutes)		
	0	180	405
Control, containing 0.2 per cent oxalate	0.347	0.350	0.357
Glucose added up to 0.5 per cent	0.344	0.344	0.354
Glucose added up to 2.0 per cent	0.343	0.343	0.334
Sodium citrate added up to 0.5 per cent	0.342	0.344	0.348
Sodium citrate added up to 2.0 per cent	0.339	0.317	0.327

TABLE 8

The effect of injecting glucose and citrate into the stagnant organs

	M.C.F. IN NaCl GRAM/100 ML.		
	Duration of stasis (minutes)		
	0	120	240
Spleen, rostral part, control	0.321	0.346	0.372
Spleen, caudal part, 9 ml. 5 per cent citrate injected	0.321	0.326	0.320
Right kidney, control	0.321	0.328	0.414
Left kidney, 8 ml. 10 per cent glucose injected	0.322		0.305

Mean corpuscular volume. Another approach to the problem is to see whether the mean corpuscular volume changes under the condition of stasis. One should expect to observe an expansion of cell volume in the stale blood if there is an unequal diffusion of water in an inward direction as a consequence of an increase of osmotic concentration within the cell. Our results confirm this deduction.

The hematocrit value and r. b. c. count of the stale oxalate (0.2 per cent) blood with and without previously adding glucose were determined in two

experiments and the mean corpuscular volume (M.C.V.) was calculated according to the conventional method. The determination of the cell volume per cent was carried out by means of the modified Van Allen hematocrit tube as described by Wu and Tsai (1940). The results of these two experiments consistently showed an increase of M.C.V. with the advance of stasis. However, it did not occur, if glucose was added up to 2 per cent before stasis treatment; as a matter of fact, the cell as revealed by microscopic examination shrank under this condition. The data given in table 9 suffice to establish the above points.

TABLE 9

The influence of stasis on the mean corpuscular volume

DURATION OF STASIS	R.B.C. (MIL./CMM.)		CELL VOLUME (PER CENT)		M.C.V. (μ^3)	
	Control	Glucose added	Control	Glucose added	Control	Glucose added
hours						
0	6.35	6.20	46	47	72.3	75.3
3	6.25	6.45	48	44	76.7	68.2
6	5.98	6.57	53	43	88.7	65.5

DISCUSSION. Bergenhem and Fahraeus (1936) claimed to have demonstrated the presence of lysolecithin in the defibrinated or citrated blood kept for several hours at body temperature. They believed that spherocytosis in the stale blood is due to the adsorption of lysolecithin on the cell surface. Since spherocytosis is always a concomitant phenomenon of increased fragility and a predisposing condition of hemolysis, one may postulate the decreased osmotic resistance of the stale blood as a consequence of lysolecithin formation in the plasma. However, this assumption is not substantiated by our present finding because we have demonstrated that the old plasma, which, according to the above view, should contain ample lysolecithin, does not increase the fragility of the fresh washed cells subjected to stasis.

From the results described in the present report, it appears very likely that the increased fragility of the red cells induced by stasis is due to the accumulation of osmotically active metabolites in the interior of the cell which causes a transference of water from the plasma into the cell and thereby makes the cell enlarge and become more spherical, leading to increased fragility and hemolysis. In a number of experiments where the plasma and the packed cells were kept separately, we have shown that the rate of lactic acid formation in the cells during stasis is definitely faster than in the plasma. The results of one of such experiments is presented graphically in figure 1. This seems to furnish strong evidence supporting the assumption regarding the accumulation of metabolites within the cell. One must of course bear in mind that lactic acid is not the only one of the metabolic products that increases the osmotic concentration in the cell interior. Although we did not study the formation of other osmotically active substances there is every reason to believe that they would exert a similar effect.

The favorable action of washing may be explained by two alternative theories. 1. It may be ascribed to the removal of glucose and other metabolic substances whose breakdown results in an increase of osmotic concentration within the cell. Addition of glucose to the washed cell suspension may start glycolysis again, but the subsequent change of osmotic condition within the cell is counterbalanced by the glucose and its breakdown products in the cell environment whereby the increase of fragility is hindered. 2. The process of washing may in some way make the cell membrane more permeable to osmotically active substances so as to set free the cell from the accumulation of them. This explanation accords with the fact that the reintroduction of glucose into the cell suspension does not cause the cell to return to its former condition of being susceptible to stasis effect. On the same assumption one may explain the deprivation of the old

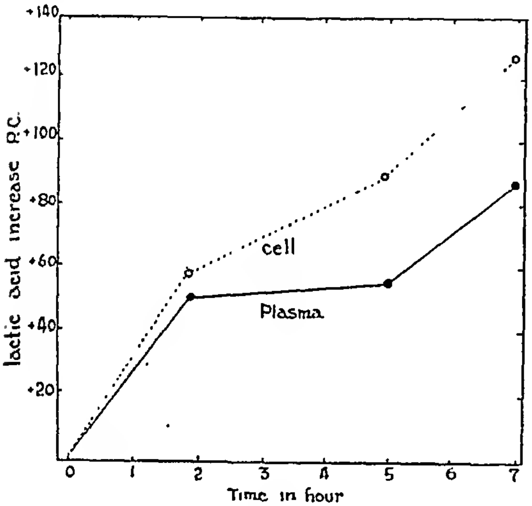

Fig. 1

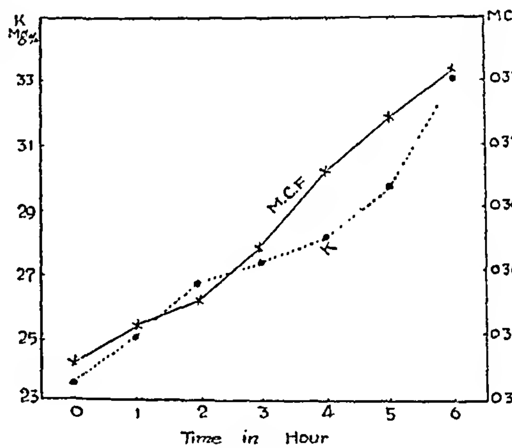

Fig. 2

Fig. 1. Showing the relative rate of lactic acid formation in the plasma and the cell during stasis.

Fig. 2. Increase of plasma K-content during stasis of the blood.

fragile cell from the ameliorating effect of washing as being due to the loss of changeability of membrane property.

The above discussion immediately leads us to the consideration of the possibility of alteration of membrane permeability during stasis. The question is far more complicated than appears at first because a change of permeability to one substance does not necessarily mean a change of permeability to other substances. We have carried out several experiments in which the plasma potassium of the blood after varying periods of stasis was estimated according to the method of Kramer and Tisdall (1921). In all four experiments performed we have found that the potassium content of the plasma increases with the duration of stasis. This is in accord with the finding of Scudder (1939) and of Downman, Oliver and Young (1940) on stored blood. When the average K-contents and the M.C.F. values are plotted together against time of incubation (fig. 2)

it reveals the fact that the rise of potassium level runs almost parallel with that of erythrocytic fragility. The shift of this original impermeable kation from the cell to the plasma clearly indicates the increased permeability of cell membrane to this ion, though the permeability to other ions or molecules may not follow the same change. At any rate, the alteration of membrane permeability still remains as a possibility of primary or secondary importance in the causation of increased fragility by stasis.

The hypertonicity of the plasma may produce a double effect. In the first place, it causes shrinkage of the cell and thus delays spherocytosis. In the second place, it tends to balance up the increasing osmotic concentration of the cell during stasis, hence avoiding the outweighing inward diffusion of water and expansion of cell volume. The increase of M.C.V. and the diminution of cell volume by addition of sufficient glucose justify the above interpretation. But again, the possibility of change in membrane permeability has not been ruled out in this case.

SUMMARY

In the present investigation various experiments were designed to elucidate the mechanism underlying the increase of erythrocytic fragility induced by artificial stasis. It was found that the phenomenon of increased fragility does not occur if the cells were previously washed with buffered saline. It is also absent in the blood to which sufficient quantity of glucose or citrate has been previously added so as to make the plasma highly hypertonic. The above findings may be explained by the rapid formation and possible accumulation of osmotically active metabolites within the cell. Lactic acid is one of these substances, but probably not the only one because addition of this substance to the blood before incubation does not increase fragility to the extent anticipated if it were the sole agent. The possibility of alteration of membrane permeability by stasis which is not excluded in the present experiment is also discussed.

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CHEMICAL CHANGES IN THE RABBIT HEART DURING HYPERTROPHY[1, 2]

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The present paper reports the results of chemical analyses of rabbit hearts made at intervals after the rupture of an aortic valve leaflet. Such hearts undergo a rapid hypertrophy. It is of interest, therefore, to compare the chemical changes in this tissue with those in other tissues undergoing rapid growth. Moreover, chemical differences between the normal and hypertrophied myocardium might be expected to result from the decreased capillary concentration (1) and consequent longer pathways of diffusion of nutrients and metabolites, of the latter.

Experimental methods. *Material.* New Zealand white male rabbits weighing about 2.2 kgm. were used for the greater number of the experiments reported.

Preparation of experimental animals. An aortic valve leaflet was ruptured by means of a sound introduced under aseptic conditions through the carotid artery (1). A sham operation, through all steps except the rupture of the valve, was carried out on an *operated control* group of animals. These hearts were taken for analysis 3 days after operation.

Preparation of tissues for analysis. Twenty milligrams of sodium pentobarbital, per kilo of body weight, were administered intravenously. Blood was drawn and defibrinated under oil. The injection of anesthetic was repeated. The heart was removed while beating, trimmed, blotted off and weighed to the nearest decigram. The left ventricle and interventricular septum were dissected out and used for analysis. The aortic valves and surrounding tissues were kept intact, and the insufficiency of the valve was confirmed for each hypertrophied heart.

Chemical methods. The methods of Hastings and co-workers (2, 3, 4) were used except as noted.

The amount of *blood* in the tissues was determined by extracting the hemoglobins with 10 volumes of 0.4 per cent ammonia water for 24 hours at 4°. The total hematin was estimated and a correction was made for myoglobin as determined by Watson's method (5).

Sodium was determined in the *potassium* chloroplatinate (6) filtrate. In order to avoid the tendency of the precipitate to float when centrifuged, and supersaturation (4), the use of alcohol to induce precipitation (7) was adopted.

[1] A report of this work was presented at the meeting of the American Society of Biological Chemists at Boston in March, 1942. Fed. Proc. 1: 116, 1942.

[2] This work was made possible by a grant from the Commonwealth Fund.

Phosphorus fractions. Acid soluble phosphate (AP) was determined by the extraction of about 0.5 gram of tissue with 10 cc. of 0.75 N nitric acid per gram, for 2 hours. The mixture was centrifuged and the residue was extracted successively for hour periods with 10 cc. per gram of 1, a mixture containing equal volumes of 95 per cent ethanol and diethyl ether; 2, a mixture of 10 volumes of alcohol and 90 volumes of ether, and 3, finally was extracted overnight with the same volume of petroleum ether. The combined extracts comprise the *lipid phosphorus* fraction (LP), while the residue is designated the *nuclear phosphorus* (NP). From the amount of phosphorus found in the lipid extract there was subtracted a correction for the amount of acid soluble phosphorus left in contact with the residue from the acid extract.

Calculations and assumptions. The assumptions, methods of calculation and symbols of Hastings and Eichelberger (2) were followed in the description of the results of analysis.

In order to make use of the heart weight-body weight ratio as a measure of the degree of hypertrophy the analytical figures have been treated in certain calculations as if they were representative of the whole heart. Since the tissue analyzed is a rather constant fraction of the whole (8) (9) such calculations should be valid for comparative purposes.

RESULTS. The results of the analyses of 85 rabbit hearts are summarized in table 1. Each value represents the average of analyses of the number of individual hearts shown in the second column. The first 2 lines of table 1 show the chloride, water and phase data for two control series, 8 normal and 6 operated controls. The 2 series do not differ significantly in any particular. The third line shows the combined average values of all control animals. The average ratio of heart weight to body weight, 2.22 grams per kgm., may be compared with the values 1.97, 2.16 and 2.25 reported by Herrmann and collaborators (8, 10).

The next three lines of table 1 give the results of analyses made 1, 2, or 3 days, respectively, after operation. A moderate increase in heart weight is to be noted. Chemically, the outstanding difference between these and normal hearts is to be found in the elevated water and extracellular electrolyte content of the hearts of the operated groups. This is reflected in the chloride, sodium and extracellular phase averages. For example, on the third post-operative day the average extracellular phase was found to be 280 as compared with an average normal value of 241. It is also apparent in the values for tissue solids and nitrogen.

The magnitude of the changes involved is brought out more clearly by reference to the data for the analyses of the individual hearts (table 2). The highest value for the $(F)_{Cl}$ found in any normal heart was 264. In 2 of the 4 hearts analyzed 1 day after operation, and in 1 of 3 hearts analyzed 2 days after operation the values were greater than this highest normal value. On the third post-operative day, the extracellular phase was greater than 270 grams per kgm. in 8 of the 14 hearts analyzed and, with 1 exception, the value was greater than the normal average in each of the remaining hearts.

TABLE 1

Average analyses of normal and hypertrophied rabbit hearts

Analyses are referred to 1 kilo of blood-free, fat-free tissue or phase

DESCRIPTION	NUMBER OF EXPERIMENTS	H.W./B.W.	Cl	F_{Cl}	S	$\{H_2O\}_C$
		grams/kgm.	*m eq.*	*grams*	*grams*	*grams*
Control	8	2.19	27.7	245	214	720
Operated control	6	2.25	26.4	235	210	722
All controls	14	2.22	27.2	241	212	721
σ		0.13	2.2	18	4	8
P.O. 1 day	4	2.40	32.1	275	209	715
P.O. 2 days	3	2.49	28.9	257	198	737
P.O. 3 days	14	2.69	32.1	280	198	729
σ		0.34	3.9	31	10	10
P.O. 4–6 days	7	2.61	27.8	239	202	738
σ		0.28	1.2	6	5	7
P.O. 7 days	7	2.81	26.5	231	201	742
σ		0.26	2.1	16	5	9
P.O. 8–9 days	3	2.61	27.4	238	210	726
P.O. 13–16 days	6	3.25	23.3	205	204	746
σ		0.24	3.3	25	7	9
P.O. 19–24 days	5	3.62	24.6	220	211	737
σ		0.43	1.7	18	5	9
P.O. 31 days	5	3.36	23.0	207	204	746
σ		0.29	1.8	11	4	6
P.O. 64 days	3	3.11	24.7	208	212	736
P.O. 80 days	1	3.85	22.1	195	210	742
P.O. 138–147	6	3.72	27.3	232	202	741
σ		0.35	1.4	14	3	5
P.O. 152–257	7	3.13	25.9	219	201	744
σ		0.47	1.6	11	5	6

Symbols used: H.W., heart weight; B.W., body weight; F, extracellular phase; $\{H_2O\}_C$, water per kilo intracellular phase; AP, acid soluble phosphorus; LP, lipid phosphorus; NP, residual phosphorus; S, tissue solids; σ, standard deviation; P.O., post-operative.

TIME AFTER OPERATION	NUMBER OF EXPERIMENTS	Na	F_{Na}	K	AP	LP	NP	N*
days		*m eq*	*grams*	*m eq.*	*mM*	*mM*	*mM*	*grams*
Control	6	35.4	247	82.5	41.8	27.8	11.1	28.4
		2.7	17	1.7	2.3	2.2	1.4	0.7
3	8	42.1	303	77.4	35.1	26.8	10.5	26.7
		3.6	29	2.0	2.7	1.9	1.2	1.2
7	4	35.1	247	79.4	37.4	28.4	10.6	27.2
13–15	4	30.9	224	79.2	36.7	26.5	10.8	27.5
31	4	33.4	239	80.0	39.3	26.2	10.3	28.1
64	3	31.0	215	73.7	42.2	27.7	10.5	
138–147	6	35.3	245	74.9	38.9	24.3	10.8	27.8
		2.0	18	3.1	3.1	2.5	1.2	0.9
152–257	7	37.0	257	75.1	39.0	25.2	9.3	27.6
		3.1	21	2.3	2.3	2.1	0.8	1.0

* Not corrected for blood content of the tissue.

None of the hearts analyzed on the fourth or subsequent days was found to have an extracellular phase in excess of the proportion found in the normal hearts. An interpretation of these results is found in the assumption that there exists a period of extracellular edema of considerable magnitude but of relatively short duration. Because the edema is transient, it is not always possible to demonstrate it, for the time of its appearance may not coincide with the time chosen for analysis.

While the major part of the increased heart weight on the third post-operative day can be attributed to an extracellular edema, there is also demonstrable at that time an increase in the intracellular phase. This is brought out in figure 1 in which the weight of intracellular phase per unit of body weight: $\frac{\text{H.W.}}{\text{B.W.}}\left(1 - \frac{F_{Cl}}{1000}\right)$ has been plotted for each period studied. Cell growth appears

TABLE 2

Analyses of hearts three days after valvulotomy

Analyses are referred to 1 kilo of fat-free, blood-free tissue

H.W./B.W.	F_{Cl}	S	$\{H_2O\}_C$	F_{Na}	K	AP	N*
grams/kgm.	*grams*	*grams*	*grams*	*grams*	*m.eq.*	*mM*	*grams*
2.92	268	210	716	281	75.9	42.0	27.5
3.01	250	194	745	278	79.8	35.2	27.8
3.03	306	185	741	310	74.9	32.7	25.1
3.00	287	212	707	285	76.5	34.4	27.2
3.06	321	183	735	347	74.6	34.3	24.8
2.43	262	194	740	284	79.4	34.8	26.5
2.47	275	202	726	288	78.9	32.7	26.4
2.43	250	204	731	250	79.2	34.6	28.0
2.46	260	209	722				
2.68	309	192	727				
3.32	354	178	729				
2.28	231	202	741				
2.33	275	206	719				
2.22	273	201	727				

* Not corrected for blood content of the tissue.

to proceed in a rather regular fashion for about 3 weeks. Further increase during longer intervals could not be clearly demonstrated.

As the growth of the intracellular phase continues there is a tendency for the proportion of this phase in the tissue to reach a value somewhat greater than normal. This is brought out in the extracellular phase data for the 13 to 16 day, and subsequent, periods. Thus the average $(F)_{Cl}$ for all hearts analyzed from 3 to 80 days after valvulotomy is 209, σ 12, compared with the normal value of 241, σ 18. The elevated proportion of intracellular phase tends to magnify the tissue content of all intracellular substance. This tendency is counterbalanced, however, by the higher water content of the intracellular phase ($\{H_2O\}_C$) of all the hypertrophied hearts.

The chemical changes in the intracellular constituents which occur during hypertrophy are made apparent by calculating the composition of the "increment of growth" for each period studied, by means of an equation similar to that of Yannet and Darrow (28) $K\Delta = \frac{C_H K_H - C_N H_N}{C_H - C_N}$, where C represents the intracellular phase in grams per kilogram of body weight; K the composition of the intracellular phase with respect to a given constituent, and the subscripts Δ, H, and N refer to the increment of growth, the hypertrophied heart and the normal heart, respectively. The results of the calculations are presented in table 3.

For each series of experiments the composition of the intracellular phase is given in the first line and the composition of the increment of growth on the second. The data of the third line represent the composition of the increment of

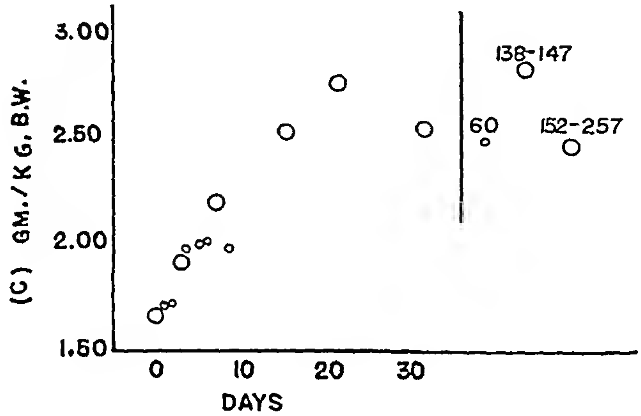

Fig. 1. Increase of intracellular phase. Large circles represent averages of 5 or more analyses; small circles 2 or 3 analyses. The ordinates represent the intracellular phase in grams per kilo of body weight.

growth referred to a normal intracellular solid content, i.e., $K\Delta \text{ corr.} = K\Delta \frac{(1000 - \{H_2O\}_C)_N}{(1000 - \{H_2O\}_C)_\Delta}$.

When the concentrations of cellular constituents are compared on the basis of the same quantity of cell solids, it is seen that in general the concentrations are similar to those of normal tissue. However, the acid soluble phosphorus concentration appears to be less than normal during the early periods, but to reach a normal level after some weeks and to be maintained at this level throughout the period studied. In contrast, potassium and phospholipin phosphorus are present in less than normal concentrations at the longer periods.

The difference in intracellular water concentration between hypertrophied and normal hearts is much too large to be accounted for on the basis of a possible difference in connective tissue content (see e.g., (3) (11)). Moreover, no significant difference in collagen nitrogen was found. The average collagen nitrogen (12) of 5 normal hearts was found to be 5.88 grams of nitrogen per kilo, σ 0.78, compared with 5.09, σ 0.91, for 16 hypertrophied hearts.

An occasional experimental animal died of what appeared to be congestive heart failure with anasarca. It was never found possible to bring such a specimen to analysis *ante mortem*, but the analysis, *post mortem*, of 1 specimen is shown in table 4 (expt. 100). This animal was found dead 82 days after operation and was stated to have been alive 17 hours earlier. Analysis revealed a very high water content, sodium and chloride concentrations greater than twice normal, and a potassium concentration about 70 per cent of normal.

TABLE 3

Composition of increments of growth of intracellular phase

Analytical values are referred to 1 kilo of intracellular phase

	TIME AFTER OPERATION	INCREMENT	H_2O	K	AP	LP
	days	*grams/kgm. B.W.*	*grams*	*m.eq.*	*mM*	*mM*
	Normal	0 25	721	108	54.6	36.3
A	3	0.25	729	114	49.6	37.2
B			780	134	21.2	43.2
C				170	26.9	54.8
A	13–15	0.90	746	102	46.2	33.4
B			792	85	32.3	28.0
C				114	43.3	37.6
A	31	0.92	746	101	49.5	33.0
B			792	83	42.0	27.0
C				111	56.3	36.2
A	138–147	1.16	741	99	50.7	31.6
B			776	82	46.5	24.8
C				99	55.9	29.8
A	152–257	0.76	744	96	50.0	32.3
B			794	64	42.0	23.4
C				86	56.8	31.7

A—Composition of intracellular phase of heart.
B—Composition of increment of growth of intracellular phase.
C—Composition of increment corrected to normal solid content.

The results of experiments designed to determine the chemical changes which occur in the myocardium *post mortem* are given in the final 6 columns of table 4. In each instance 50 mgm. per kilo of sodium pentobarbital were injected intravenously. Except as indicated (expt. 141) respirations ceased in about 5 minutes, and the heart failed about 5 minutes later.

The water content of the myocardium rises rapidly during failure under anoxic conditions (expts. 140, 141). The sodium and chloride content rise somewhat, but much greater increases in extracellular electrolytes appear to occur when the heart is allowed to remain *in situ* after death. Similarly, losses in potassium

and phosphorus appear to occur only during *post mortem* autolysis. These findings suggest that the loss of intracellular electrolytes occurs not only as a result of exchanges across cell membranes, but also as a result of a mechanical expression of fluid from the tissues as *rigor mortis* sets in.

DISCUSSION. The data presented here show that within a short period after aortic regurgitation is produced experimentally, the myocardium takes up considerable amounts of sodium chloride and water. An increase in the extracellular phase of heart muscle amounting to 10 per cent was found by Hastings et al. (13) to follow a short ligation of a coronary artery. This was believed to be the result both of an increase in interstitial fluid and changes in permeability due to injury. It appears that the increase in extracellular phase observed in the early stages of aortic insufficiency reflect chiefly an increase in interstitial fluid, rather

TABLE 4

Post mortem changes

Analytical values are referred to 200 grams of blood-free, fat-free solid

	NORMAL	EXPERIMENT						
		100	141†	140	137	119	133	136
H.W./B.W., grams/kgm.	2.25	4.33	1.91	2.48	2.25	2.46	3.09	2.23
Temperature, post mortem	c.18°				4°	4°	23°	23°
Time after death, hours	8	17	0	0	17	17	17	17
H_2O, grams	752	917	815	943	899	899	887	899
N*, grams	27.1	24.0	27.8	27.5	26.9	26.4	26.9	
Cl, m.eq.	25.1	56.5	28.3	31.2	36.7	32.9	47.9	47.1
Na, m.eq.	33.7	72.8	35.6	40.7	53.3	40.4	62.4	73.2
K, m.eq.	78.0	55.8	77.8	81.6	66.6	65.3	63.0	68.2
AP, mM	39.8		39.9	38.6	40.4	34.3	32.9	37.1
LP, mM.	26.5		25.2	24.9	26.7	28.3	26.0	28.3
NP, mM	11.3		11.0	10.9	8.1	11.5	9.7	9.1

* Nitrogen values were not corrected for blood content of tissue.

† The heart stopped beating 3 minutes after anaesthetic was given and was removed for analysis immediately.

than injury, for the intracellular elements undergo a rapid growth, and concentrate potassium at least as fast as is required by the synthesis of new intracellular material. The transient increase in extracellular phase and the other chemical changes in early hypertrophy are much the same as the changes which occur in other tissues when stimulated to rapid growth by means of hormones (14, 15). This suggests that such a pattern of changes may accompany the rapid growth of tissues whatever the stimulus for growth may be.

Chemically the most striking differences between the hypertrophied and the normal hearts during the intermediate periods (1 to 3 mos. postoperative) are the higher intracellular phase and intracellular water of the hypertrophied tissue. The increased intracellular phase of moderately hypertrophied hearts gives rise at times to apparent supernormal concentrations of intracellular constituents

when these are expressed per unit of tissue or, especially, of tissue solids. This tendency is apparent with respect to potassium in the analyses of many of the individual specimens of the present study, but in the averages it is obscured by the presence in each group of individuals showing the potassium loss characteristic of hypertrophy of longer duration. An increased proportion of intracellular phase presumably accounts for the rise in creatine concentration reported to occur in slight hypertrophy (16).

The composition of the hypertrophied heart 4 months or longer after operation indicates that there is a tendency for the heart to lose intracellular constituents, in these studies chiefly potassium and phospholipin. The intracellular water content remains elevated which presumably indicates the persistence of some metabolic abnormality, whether a change in hydrogen ion activity, or in the concentration of metabolically active substances in one or both phases. Concentrations of creatine, and of acid-soluble, but not phospholipin, phosphorus lower than normal have been reported to occur in the rabbit heart after several months of aortic insufficiency (16) (17). Myocardial insufficiency in the human heart is accompanied by loss of creatine, potassium and phosphorus and by increases in extracellular electrolytes and water (17) (18) (19) (20). To some extent the latter changes might be the result of ionic and water exchanges during the final stages of failure and *post mortem* if such changes in the human heart follow the pattern found in the rabbit heart.

SUMMARY

When aortic insufficiency is produced in the rabbit heart by rupturing an aortic valve leaflet, rapid changes occur in the chemical composition of the myocardium. During the first 3 days there is a transient increase of extracellular phase of considerable magnitude. The intracellular phase appears to hypertrophy at a more or less constant rate for several weeks after which further increases cannot be clearly distinguished. The hypertrophied hearts at intermediate periods are characterized by a proportion of intracellular phase somewhat greater than normal but of approximately normal composition except for an increased intracellular water content. At later periods a tendency for a loss of intracellular constituents is observed.

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REFLEXES FROM THE LIMBS AS A FACTOR IN THE HYPERPNEA OF MUSCULAR EXERCISE[1]

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It has been known for many years that in the two commonest and most important physiological emergencies calling for increased pulmonary ventilation, viz., muscular exercise and anoxemia, the hyperpnea is not referable to an increase in the amount of any known chemical stimulant in the arterial blood. Various suggestions have been advanced to explain this discrepancy, such as the existence of an unknown chemical excitant (3, 9), inadequacy of existing chemical methods to detect the responsible acid change in the arterial blood (5), or an acid shift within the respiratory center though not in the arterial blood (4, 24), but the view most widely held until recently was that both hyperpneas are brought about by an increase in the sensitivity of the respiratory center to its normal chemical stimulus (12, 16). The brilliant studies of Heymans and his collaborators (10, 11), amply confirmed by others (see 19), have shown that this increased sensitivity during anoxemia actually is brought about by excitatory nerve impulses from the carotid and aortic chemoreceptors, which furnish an adequate explanation for the coexistence of hyperpnea, hypocapnia, and alkalosis during anoxemia. In view of this course of events with regard to one of these physiological emergencies it is reasonable to suppose that excitatory reflexes may also be prominently involved in the other, but so far little attention seems to have been given to this possibility. Yet Harrison and his collaborators (6, 7) concluded from experiments on men and dogs that reflexes aroused by movements of the limbs play a part in the hyperpnea of exercise, and Alam and Smirk (1) presented evidence that a chemosensitive reflex system capable of stimulating the vasomotor center is present in the extremities of man. In view of their potential importance it is rather surprising that these experiments have not been repeated. Since Harrison's results were not very striking and since Alam and Smirk present no data bearing on the respiration, we undertook to repeat both sets of experiments from the standpoint of a possible explanation for the hyperpnea of muscular exercise.

1. *Experiments of the Alam and Smirk Type on Man.* These were performed on healthy male subjects (staff members, medical students, and technicians) reclining on a couch. A comfortable rubber face mask fitted with valves was used and the expired air was passed through a gas meter for measurement. A pneumogram was also made. Blood pressure was measured at frequent intervals in the left arm by the Riva-Rocci method and another

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cuff was applied to the right arm so that the arterial inflow could be cut off when desired. Exercise of the right forearm and hand was brought about by flexion of the fingers in time with a metronome beating once per second, each effort raising a weight of 1360 grams a distance 5 to 7 cm. With certain exceptions which will be indicated, a complete experiment comprised five periods, each lasting two minutes: *1*, resting control (two minutes of observation after a steady state had been shown to be present); *2*, occlusion of the circulation

TABLE 1

SUBJECT	PER CENT CHANGE IN																SENSATIONS			
	Resp. rate				Resp. min. vol.				Systolic B-P				Diastolic B-P							
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
P. D.	−14	−3	0	+24	0	−12	+23	+35	0	−2	+5	+9	0	0	+14	+16	0	0	0	T
	−18	0	0	0	0	0	0	0	0	0	+8	+6	0	0	+6	+6	0	0	0	0
	−10	0	−12	−16	+12	0	+16	+16	0	0	+6	+14	0	0	+2	+2	0	0	0	0
J. C	+5	0	+14		+50	−8	+61		+2	0	+16		+15	0	+6		0	NT	WN	
	+8	0	+18	+9	+15	+5	+16	+23	0	0	+8	+11	+3	0	+7	+15	0	0	P	PN
	+8	0	+40	+20	+13	0	+30	+20	+5	+2	+5	+9	+8	+2	+6	+10	0	0	WP	
	+25	0	+30	+10	+30	0	+45	+20	0	0	+2	0	0	0	+9	+16	0	0	W	NT
	+41	0	+25	+20	+37	0	+40	+61	0	0	+2	+13	+5	0	+15	+12	0	0	ND	N
	+31	−4	+7	0	+25	−13	+28	+12	+2	0	+3	+9	+3	0	+4	+6	0	0	W	DT
J. R.	+15	0	−27	0	0	−14	−16	0	0	0	+4	+6	+5	0	+9	+12	0	N	WP	N
	−12	−12	−11	+11	−10	0	+15	+25	0	0	+4	+6	0	0	+17	+17	0	0	NP	PP
	0	0	0	0	0	0	+75	+30	0	0	+10	+4	0	0	+6	0	0	0	0	0
R. E.	+40	+4	+9	−9	+40	+14	+32	0	0	0			0	0			0	0	NP	P
	0	0	0	0	+36	+12	+84	+26	0	0	+4	+6	0	0	0	+5	0	0	P	NPT
O. H.	0	0	+20	+20	0	+25	−22	+12	0	0	+4	0	0	0	+3	0	0	D	0	PT
	+47	+23	+62	+10	+30	0	+29	−3	0	0	+2	+2	0	0	+5	+5	0	0	0	0
C. S.		−11	+55	+22	0	0	+33	+7	0	0	+3	+5	0	0	+5	+5	0	NT	W	NT
J. W.	−11	0	0	−12	−8	0	+13	+3	+7	0	+10	+12	+15	0	+19	+19	0	T	T	P
H. P.	+11	−20	+16	−5	+10	+12	+31	0	+13	+2	+10	0	+12	+7		0	0	T	NP	NP
J. S.	+33	0	+33	0	+20	0	+28	0	+9	0	+6	+6	+2	0	+21	+21	0	T	WT	T
F. H.	+8	0	+15	0	+11	0	+7	+3	0	0	+2	+4	0	0	+3	+3	0	0	0	0
I. S.	+4	0	+9	+13	+27	+2	+35	−4	0	0	0	+5	0	0	0	+4	0	0	P	P
Average..	+10	−1	+14	+6	+14	+0.4	+28	+14	+2	0	+6	+6	+3	0	+7	+9				

1 = change from control to exercise alone.
2 = change from control to ischemia alone.
3 = change from control to exercise plus ischemia.
4 = change from control to continued occlusion after exercise.
Sensations: D, discomfort; N, numbness; P, pain; T, tingling; W, weakness; 0, none; blank, not recorded.

in the right arm by inflation of the cuff by a pressure higher than the systolic arterial level; release and recovery to normal, then *3*, exercise of the right forearm and hand with circulation intact; rest and recovery, then *4*, repetition of the exercise with the circulation cut off (terminated as soon as any unpleasant sensations were experienced, often in less than two minutes); *5*, cessation of the exercise without restoration of the circulation. This report is based on the results of 23 satisfactory experiments of this type on 11 subjects. The results are summarized in table 1.

The *respiratory* findings at the various stages of these experiments justify brief comments. First, since simple ischemia did not cause hyperpnea, it follows that there are in the forearm and hand of man no chemoreceptors comparable in sensitivity to those in the carotid bodies, which are strongly stimulated by total ischemia of considerably less than two minutes' duration (23). Second, voluntary exercise, even of a relatively small mass of muscle, caused a distinct (average 14 per cent) increase in respiratory minute volume even with intact circulation. Third, exercise in the presence of ischemia brought about a much greater (average 28 per cent) increase in pulmonary ventilation, which might have been due either to a specific reflex aroused by chemical substances acting locally in the muscles (since their escape was prevented by the inflated bandage), or to a nonspecific reflex of the pain type. Our findings point toward the latter explanation, because in 15 of the 22 experiments respiration returned toward normal when the exercise was discontinued even though the ischemia persisted and the concentration of chemical stimuli could not have diminished. That pain was the dominant factor here was further suggested by the variability of the response of a given individual at different times (which is difficult to reconcile with the requirements of a specific reflex system subserving an important function), and by the fact that, in all of the five instances in which the hyperpnea was greater during the final period of ischemia after exercise, pain also persisted; all of the other subjects felt more comfortable as soon as they ceased exercising.

Our findings with regard to the blood pressure seem to us to be without significance. They are much less striking than those reported by Alam and Smirk (1). Whether this is because our subjects were not exposed to as severe pain as theirs, or because we did not happen to encounter any of the unusually reactive individuals who comprised only one-fourth of those tested by Alam and Smirk, we are not prepared to say.

In order to exclude the influence of pain and other psychic factors we next undertook a series of experiments on anesthetized animals in which observations could be made along the same general lines but under more controllable conditions.

2. *Experiments of the Alam and Smirk Type on Animals.* Our purpose was to test the effects of exercise alone, of ischemia alone, and of exercise plus ischemia. To induce "exercise" without direct sensory involvement we resorted to stimulation of ventral spinal roots.

A. *Cats.* The animals were narcotized by chloralose (0.05 gram per kgm. intravenously) or barbital sodium (0.35 gram per kilo intraperitoneally). The ventral lumbar spinal roots were exposed by Sherrington's method (20), cut free on the central side, and placed on insulated electrodes raised above the cord to obviate escape of current. The stimulus was obtained from a thyratron stimulator, the frequency being 4 per second throughout and the strength adjusted so as to elicit maximal muscular activity. In some cases only the 7th lumbar pair of ventral roots were stimulated, in others the 6th lumbar, and in a few the first sacral also, were added. Respiration was recorded by a small oxygen-filled spirometer to which the animal's tracheal cannula was connected through a canister containing soda-lime. Blood pressure was registered from a carotid artery by means of a mercury manometer, 25 per cent $Na_2S_2O_3$ serving as the anticoagulant.

We made 29 observations of the effects of "muscular exercise" thus induced in 15 cats, and in every case there was some increase in respiratory minute volume. Other noteworthy results were the following: First, the stimulation involved slight to moderate increase in *depth* of breathing, rate being increased seldom and never markedly; second, there was a latent period of at least 15 seconds between the start of the stimulation and the onset of the increased depth of breathing; third, the depth increased progressively to reach a plateau; and finally, recovery was gradual and slow after the stimulus was withdrawn. Ischemia alone (produced by clamping the femoral arteries and veins or the abdominal aorta and vena cava) produced no changes in respiration. "Exercise", tested 14 times in the presence of ischemia, regularly had less effect on breathing than it had when the circulation was intact, and often it had no effect at all. When the vessels were reopened, however, hyperpnea promptly appeared. Blood pressure commonly fell during the "exercise"; it never rose even when the vessels were closed during the "exercise" period.

Characteristic examples of these findings are shown in figure 1. The results strongly suggest that the hyperpnea of "muscular exercise", induced in the cat by stimulation of ventral spinal roots, is largely due to liberation into the blood stream of chemical products which act either on the center or on chemosensitive nerve endings elsewhere than in the leg. This was confirmed by the effect of transection of the spinal cord in the lower dorsal region in 5 cats. After that operation "exercise" still increased breathing much as it had done before. If there is any peripheral reflex component in the hyperpnea of this type of "exercise" in the cat, it must therefore be small.

These results with cats are quite unlike those obtained in man, in whom the hyperpnea of voluntary exercise was definitely increased by ischemia and therefore could not have been due to direct stimulation of the center by chemical substances. This suggested that species differences may exist.

B. *Dogs.* These were prepared in the same way as the cats. The anesthetic was sodium barbital (0.25 gram per kilo intravenously) or chloralose (0.03 gram per kilo intravenously after 2 mgm. of morphine per kilo subcutaneously).

Out of 39 tests of ventral root stimulation in 11 dogs, 35 showed definite respiratory stimulation, the increase in minute volume varying from 8 to 200 per cent and averaging 62 per cent. In one there was no change (only one root was stimulated here) and in 3 there was pure depression of breathing, for which we have no explanation. The noteworthy features were as follows: First, the stimulant effect was mainly on the *rate* of breathing; depth might be unchanged, slightly increased, or slightly decreased. Second, the polypnea came on immediately with the start of the stimulation and was maximal within the first 30 seconds, after which it often diminished during the remainder of the period of stimulation. Third, when the stimulus was withdrawn breathing returned to normal almost immediately. Fourth, the response was unaltered when the femoral vessels were occluded during the stimulation, and there was no consistent effect on breathing when the vessels were reopened after the stimulation. Finally, transection of the spinal cord in the lower dorsal region completely abolished this polypnea

in each of 4 dogs on which the point was tested. Section of the dorsal lumbar roots likewise abolished the effect in other animals.

Typical examples of the dog experiments are shown in figure 2.

That these findings were not due to escape of current to the dorsal roots or to the cord itself was shown by the following: First, the polypnea did not increase further when the strength of the stimulus was increased above the level that sufficed to produce maximal muscular activity. Second, elevation of the electrodes to the fullest extent permitted by the cut ventral roots had no influence on the results. Third, the response was entirely lacking when the stimulation was repeated after the corresponding spinal nerves had been cut outside the vertebral canal; under these circumstances movements of the legs were also lacking, but escape of current into the dorsal roots or cord should not have been prevented.

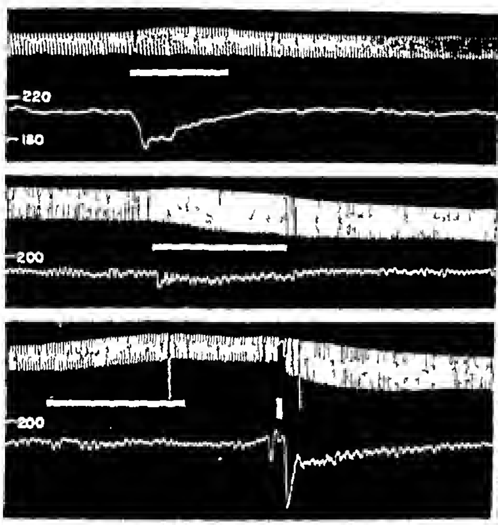

Fig. 1

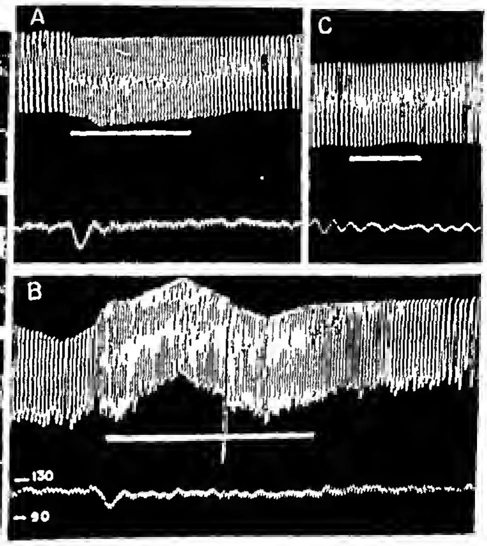

Fig. 2

Fig. 1. Cat. Chloralose anesthesia. In this and the following kymographic tracings upper record is spirometric tracing of respiration and lower record represents carotid blood pressure. Horizontal white bar indicates period of electrical stimulation of cut peripheral ends of L 7 anterior spinal roots. Numbers at left show actual level of blood pressure. Upper tracing—intact cat. Middle tracing—cord has been sectioned at level of emergence of L 4 roots. Lower tracing—cord cut, abdominal aorta and inferior vena cava clamped at start of record—released at vertical white bar.

Fig. 2. Dog. Morphine and chloralose anesthesia. Arrangement as in figure 1. L 6 and 7 anterior spinal roots stimulated. A, Intact dog. B, Abdominal aorta and inferior vena cava clamped at start of record (irregularities in spirometric tracing are due to manipulations of inflow of oxygen into the system). C, Cord cut at level of emergence of L 4 roots (vessels open).

We conclude that the respiratory stimulation associated with "exercise" induced by ventral root stimulation in the dog, unlike the corresponding effect in the cat, is essentially due to afferent nerve impulses from the limbs. Our next step was to determine whether these impulses arose from proprioceptors

responding to changes in length or position, or from chemoreceptors responding to changes in muscle metabolism. The latter seemed unlikely because the response was not appreciably enhanced by ischemia during the stimulation, but the possibility remained that adaptation (or actual deterioration in reactivity) might occur when the ischemia was as nearly absolute as it was in these experiments.

3. *Attempts to Demonstrate Chemoreceptors in the Limbs.* These experiments were of three general types: *a*, crossed perfusions in which blood from a donor animal was circulated through one or both hind limbs of the recipient by means of a perfusion pump; *b*, auto-transfusion experiments involving injection into one femoral artery of blood collected from the opposite femoral vein during "exercise", ischemia, and "exercise" plus ischemia on the side of collection; *c*, intraarterial and intramuscular injection of various chemical substances known to be associated with muscle metabolism, as well as some others. Cats and dogs were used for all three sets. It may be said at once that no definite evidence of the presence of a specific, physiologically important chemoreflex system has been obtained by any of these procedures. Detailed descriptions of the methods employed and of the individual results are therefore unnecessary, but a brief account seems desirable.

The crossed perfusion experiments were intended to test the ability of blood rendered anoxic, hypercapnic, or acidic (by appropriate manipulation of the donor) to cause a reflex hyperpnea in the recipient. No signs of any such effect were seen.

In the auto-transfusion experiments blood was collected from a branch of the femoral vein into a syringe, heparin was added to prevent clotting, and the same blood (5 to 20 cc.) was then injected into the opposite femoral artery through a suitable cannula tied into the profunda femoris, the main artery being clamped above this point during the injection. Even when the collection was made during stimulation of the muscles with the artery closed (it was opened for brief periods to permit the collection of blood from the vein), and although the appearance of the blood indicated almost complete change to reduced hemoglobin (which may be taken as an indication of the amounts of metabolic products that must have been present), there was no effect on breathing when the blood was injected into the opposite femoral artery. The same animals were responsive to intra-arterial injections of KCl (see below), so that their leg reflex systems were not unreactive.

The experiments in which various chemicals were injected intra-arterially were carried out by the same general methods as those just described. The only essential difference was the use of decerebrated cats and dogs instead of anesthetized animals because the former proved more reactive. The results were confirmatory in the main of those of the similar experiments reported by Moore and his associates (15). *Acids* (hydrochloric, lactic, phosphoric) were active when injected in concentration of 0.1 N or stronger but thrombosis of the artery frequently followed repeated injections. To determine the sensitivity to hydrogen ions we turned to buffer mixtures of sodium phosphates. They were made isotonic with NaCl and injected at 38°C. in 1 cc. dosage with the arterial inflow cut off during the injection, restored just afterward. The threshold value turned out to be about pH 6.7, which was effective only weakly and occasionally in cats. To obtain consistent and fairly strong stimulant effects a mixture of pH 6.3 or less had to be used in dogs and cats. *Potassium chloride* was the most consistently effective and repeatable of all the chemical stimulants used by us. The minimum effective dose on intra-arterial injection in cats was 0.1 mgm. in 1 cc. of warm 0.9 per cent NaCl (the latter being entirely ineffective alone), corresponding with 0.0013 M solution of KCl. This was active only weakly and occasionally; for consistent and fairly powerful effects doses of 1 to 5 mgm. in 1 cc. (0.013 to 0.065 M) were required in cats, 1 to 10 mgm. (0.13 M) in dogs). We were also able to test a number of intermediary products of muscle metabolism that were placed at our disposal by Professor Meyerhof. Positive results were obtained with a number of them but all of the active solutions proved either to contain barium (which Moore *et al.* (15) found to be highly

effective) or to be more acid than pH 6.0. We did however make some valid tests with creatine and phosphocreatine, both of which proved entirely ineffective. *Creatine* was used in 3 per cent solution in isotonic sodium phosphate buffers at pH 7.0, 6.7, and 6.5 and the amounts injected ranged from 1 to 10 cc. in decerebrated dogs and cats. *Phosphocreatine*, carefully freed of barium, was used in 0.5 per cent solution in phosphate buffers at pH 7.0 and the amounts injected (in dogs and cats) were 1 to 5 cc.

In addition to these substances, *NaCN* and *lobeline* were injected into the femoral artery and found effective only after a latent period of about 30 seconds. The inference—that the response, when it occurred, was due to an action on the carotid and aortic bodies—was confirmed by the effects after denervation of these structures. After the denervation there was no hyerpnea from doses which had previously caused a strong delayed response. *Ether* dissolved in saline was also tried and found to cause distinct hyperpnea, even in concentrations as low as 0.1 per cent. The effect came on immediately and was abolished by denervation of the limb. The implications with respect to the cause of the well-known hyperpnea of ether anesthesia (8, 17, 18) are obvious and are to be the object of further study.

Since the hydrion concentration and potassium content of muscle are known to rise during vigorous contraction, a specific chemoreflex system responding to those changes became a distinct possibility. However, when we injected these and other substances intramuscularly we found (like Moore and his collaborators (15)) that such injections were without effect. We injected warm isotonic solutions of phosphate and bicarbonate buffer mixtures, KCl, and phosphocreatine, in amounts ranging from 1 to 20 cc. and at pH ranging from 7.0 to 6.3, into the adductor, quadriceps, hamstring, gluteal, and gastrocnemius muscles of decerebrated dogs and cats, and in no case were there any definite stimulant effects, even from solutions that had strong effects when given intra-arterially.

It is difficult to conceive of a physiologically important chemo-reflex system, responsive to products of muscle metabolism, that would not be activated by any of these procedures except intra-arterial injections of foreign chemicals. We conclude that although the latter experiment shows a chemoreflex system to be present, the entire effect is probably due to pain impulses set up in or near the arterial wall and therefore has no great physiological importance. In this we confirm the findings and interpretations of Moore *et al.* (15). The increased breathing associated with ventral root stimulation, which we have shown above to be reflex in origin in the dog, therefore cannot arise from chemoreceptors. The experiments next to be described were undertaken to determine whether it is referable to proprioceptor reflexes of the type described by Harrison (6).

4. *Experiments of the Harrison Type.* These were performed on dogs and cats prepared as described above (p. 539); in addition the femora were disarticulated or transected and their proximal ends were clamped rigidly so that movements of the legs were not communicated to the trunk. The feet were tied to a rod that was moved vertically (in a few cases horizontally) by an electric motor; in all of the experiments now under discussion the rate of movement was 200 per minute and the type was intended to simulate normal running movements. Nineteen experiments of this type were performed on dogs, 12 on cats.

Dogs. A typical example is shown in figure 3. Increase in respiratory minute volume was seen at least once in each experiment during passive movements of the legs; the increase varied from 22 to 125 per cent and averaged 52 per cent. Thus we are able to confirm Harrison's (6) findings with regard to passive movements of the limbs. The respiratory effect of these movements had the same characteristics as that of active contractions induced by ventral root stimula-

tion, i.e., it was predominantly an effect on the *rate*, and the onset and recovery were both abrupt. The reflex nature of the response was proved by demonstrating both its continued presence during occlusion of the femoral blood vessels and its complete absence after section of the spinal cord or of the nerves to the hindlegs. The reflex evidently is not set up by stretching muscles or tendons because in 4 experiments traction was exerted on one or more of the tendons of the quadriceps and hamstring groups, and in no case was there any respiratory stimulation; in all these, shaking the legs still caused a typical response after all the tendons were cut through. That the knee joint is prominently concerned was suggested in 6 experiments in which continuous flexion of one knee caused distinct respiratory stimulation, and the result was unaffected

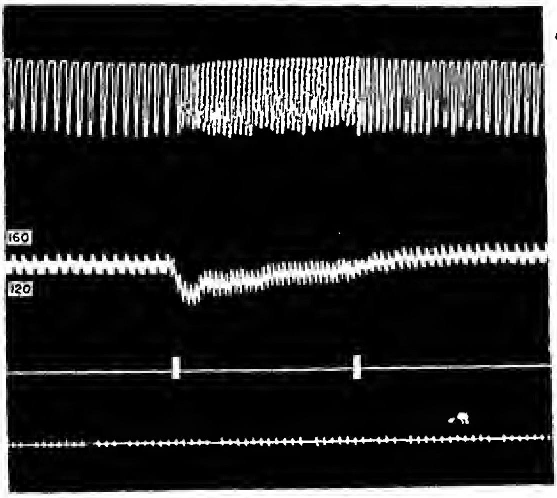

Fig. 3

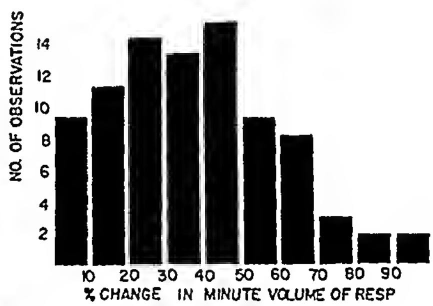

Fig. 4

Fig. 3. Dog. Anesthesia by barbital. Between signals, passive movements (200 per min.) of both hind limbs. Vessels and nerves intact.

Fig. 4. Frequency-distribution chart of respiratory response of healthy adults to passive movements (100 per min.) of left leg below knee. Each block indicates number of observations out of total of 86.

by division of all the quadriceps and hamstring tendons. Final proof of that fact was obtained by injection of 2 per cent procaine into and around the knee joint, for following this, shaking or flexion of the leg was entirely ineffective until, after about 30 minutes, the effects of the drug wore away and the response returned. In one experiment denervation of the knee was accomplished by division of all nerves in the vicinity, and this also abolished the effects of shaking or flexion. These experiments therefore show clearly that the respiratory stimulation produced in dogs by passive movements of the hindlegs is due to a reflex arising in and around the joints, particularly the knee.

Cats. The responses were both less consistent and less marked than those seen in similar experiments on dogs. As with the ventral root stimulations, the stimulant effect was mainly on the depth of breathing, it began after a latent

period, and wore away gradually after the stimulus (shaking or flexion in this case) was discontinued. Nevertheless this effect was proved to be entirely reflex by its persistence during closure of the femoral artery and vein (3 experiments) and by its absence following section of the nerves to the legs (2 experiments) or injection of 2 per cent procaine into the knee joints (2 experiments).

In view of these marked species differences, a corresponding study was carried out on man.

Healthy adult males (staff members, technicians and medical students) were used as subjects. They reclined on a padded table from which a part had been cut away so that the left leg hung free from a level about 6 inches above the knee. The left foot was tied to a stirrup on the end of a rod that was moved back and forth a distance of about 2 feet by means of an electric motor; the number of movements was 100 per minute. The subject's expired air was collected through a comfortable valved rubber face mask and was measured directly by a gas meter; a pneumogram was also recorded. The control period was usually 15 minutes (longer if a steady state had not been reached), the period of passive movement 1 to 2 minutes, and the recovery period about 6 minutes. A total of 86 tests of this sort were made in 50 subjects. The results are summarized in figure 4.

It is quite evident that the respiration of these subjects was stimulated consistently and powerfully by passive movements of one leg. Only in 4 was the increase in respiratory minute volume less than 10 per cent. In 38 it was between 20 and 60 per cent and the largest number (14 subjects) were in the group showing an increase of 40 to 50 per cent. The responses partook of the characteristics of both dogs and cats in that the increased breathing involved rate (as in the dog) and depth (as in the cat), began almost immediately (as in the dog) but often faded away gradually (as in the cat).

While the psychic factor cannot be altogether excluded in experiments such as these, the passive movements were not associated with any discomfort or pain. Direct evidence that these stimulant effects were due at least in part to afferent impulses from the leg was obtained in 4 patients in whom the experiment was carried out just before and again after the induction of spinal anesthesia. The results are summarized in table 2. The uniformity with which the respiratory response was reduced by spinal anesthesia seems to us to indicate that psychic factors were not entirely responsible. We therefore conclude that in man, as well as in the dog and cat, passive movements of the leg give rise to reflexes stimulant to respiration.

Discussion. The results of these experiments fully confirm Harrison's claims (6) (7) as to the existence of stimulant reflexes to respiration associated with movements of the limbs, but we are not prepared to accept his conclusion (6, p. 220) that: "The increase in ventilation produced by mild muscular movements is reflex in origin." We have no hesitation in affirming that these reflexes play a part, but from the data now available we are forced to conclude that the reflexes fall short in several important respects from the requirements of a complete explanation. One of these is the difference between various species of animals: we found that in the dog the reflexes aroused by passive movements stimulated the rate of breathing predominantly while in the cat the main effects

were on the depth and in man both rate and depth were stimulated. Another shortcoming has to do with time relationships: in the dog the reflex hyperpnea came on and ended abruptly, in the cat both onset and recovery were gradual, and in man the onset was usually abrupt but the recovery was frequently gradual. The findings in the cat come closest in both these respects to a satisfactory explanation for the hyperpnea of exercise, but in this animal the reflex component was relatively very weak and the stronger effects associated with active muscular contractions (ventral root stimulation) turned out to be due to direct stimulation of the center by a product of muscle metabolism (p. 539). Still another shortcoming of the reflex explanation is that it leaves no provision for adjustment of pulmonary ventilation to the work done, but only to the rate and extent to which the limbs are moved. It is common knowledge among

TABLE 2

Per cent change in respiration produced in man by passive movements of one leg

PATIENT NUMBER	BEFORE SPINAL ANESTHESIA		DURING SPINAL ANESTHESIA	
	Rate	M.V.	Rate	M.V.
1	+47 +22 +27 +20	+51 +26 +28 +39	+12.5 +6	+15 +11
2	+18 +19	+21 +29	+5.5 +6.2	+12.2 +15.8
3	+20 +20 +29 +20 0	0 +5.2 +43 +30 +22	0 +6.2 +6.7	−7 +6.6 +2.5
4	+13 +13	+40 +43	+4.5	0

cyclists that hyperpnea is much more closely related to the load than to the rate of pedaling, and a considerable mass of objective evidence to that effect is available from experiments on the bicycle ergometer (13, 16). The reflexes also have quantitative deficiencies, for the reflex effects thus far described are not nearly intense enough to justify the belief that they alone could cause the hyperpnea of muscular exercise, which is the strongest of which the organism is capable.

Final decision on these points must be deferred until other joints and other types of movement have been studied. At present we prefer to conclude that the reflexes constitute only one of several factors involved in this hyperpnea. This conclusion seems to us to be advisable in view not only of existing experimental evidence but also of the history of respiratory physiology, which has been characterized by a series of attempts, all of which have eventually turned

out to be mistaken, to explain too many observations on the basis of a single, simple theory. From the evidence now available we believe that "increased excitability" of the respiratory center during muscular exercise (i.e., hyperpnea without corresponding increase in cH or pCO_2 in the arterial blood) is probably due in part to excitatory afferent impulses from the limbs, in part to irradiation of excitation from cortico-spinal nerve fibers into the reticular formation of the medulla, and perhaps in part to afferent impulses from the lungs, aroused there by the changes in the pulmonary circulation associated with exercise. To attribute great importance to reflexes involving the pulmonary circulation is in line with the most recent trend of thought concerning the dyspnea of heart disease (2, 7), but evidence as to the applicability of this conception to normal subjects is lacking at present. Irradiation of excitations, originally suggested by Geppert and Zuntz (3), is the explanation favored by Krogh and his collaborators (12, 14); its part in the total respiratory response to exercise must necessarily be ascertained by exclusion when all other factors have been evaluated. As for reflexes from the limbs, it is possible (though in our opinion improbable) that the sum-total of all the afferent impulses aroused during muscular exercise will eventually afford an adequate explanation for the concomitant hyperpnea, particularly when there is also an increase in metabolic activity to prevent reduction in the stimulus level in the arterial blood during the hyperpnea. On the basis of *a priori* reasoning one would expect reflexes aroused in muscle chemoreceptors by accumulated products of muscle metabolism to be a much more important factor than reflexes from proprioceptors in the joints, and it is interesting to note that the first modern attempt at explaining the hyperpnea of exercise ascribed the major rôle to reflexes set up in the muscles by carbon dioxide (21, 22). Yet our results compel us to discard this attractive possibility and to conclude that, although respiration and circulation can be stimulated by reflexes aroused in the limbs by chemical substances, these phenomena are related to pain and not to a specific reflex system of physiological importance.

SUMMARY AND CONCLUSIONS

In human subjects exercise of one forearm and hand caused distinct hyperpnea, which was increased if the circulation was cut off. This potentiation by ischemia was probably due simply to pain. In analogous experiments on anesthetized dogs and cats, "exercise" of the hind-limbs (produced by stimulation of the ventral spinal roots) also caused hyperpnea, which in the dog was not influenced by ischemia but was abolished by transection of the spinal cord while in the cat it was reduced or abolished by ischemia, unaffected by cord transection. This hyperpnea therefore was due mainly to reflexes in the dog and in man, to direct central stimulation by chemical products of muscle contraction in the cat.

Various methods were used to detect a specific, chemosensitive reflex system in the limbs, without success. Passive movements however produced hyperpnea in dogs, cats and men, the effect being most marked in man and least marked in the cat. The reflex nature of this hyperpnea was proved by its absence after denervation or chordotomy in animals and by its diminution during spinal

anesthesia in man. By means of traction on muscles and tendons and of local anesthesia of the periarticular surfaces, the reflex was shown to arise largely or wholly in and around the knee joint, not in the muscles or tendons.

The possible significance of these findings to the respiratory response to muscular exercise is discussed and reasons are given for believing that while reflexes of this type unquestionably account for some of the hyperpnea, they probably cannot account for all of it.

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THE EFFECT OF BILE IN THE INTESTINE ON THE SECRETION OF PANCREATIC JUICE

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The theory that bile in the intestine promotes the absorption of secretin and, therefore, increases the secretion of pancreatic juice was proposed by Mellanby (1926). In spite of the demonstration by Lueth and Ivy (1927) and by Dragstedt and Woodbury (1934) that as much pancreatic juice is secreted when bile is excluded from the intestine as when it is present, Mellanby's theory continues to receive prominent mention in modern textbooks of physiology (Cowgill, 1941; Best and Taylor, 1940; Evans, 1941). Although the evidence just cited eliminates the possibility that bile is necessary for the secretion of a normal amount of pancreatic juice during digestion, it leaves open the question of whether bile is capable of stimulating the pancreas in the absence of other stimuli. We have undertaken to investigate the action of bile alone or of bile mixed with various food products in the intestine on the secretion of pancreatic juice in fasting, unanesthetized animals.

METHODS. Three dogs were used for most of the work but some of the results have been repeated on two additional animals. The dogs were provided with gastric and duodenal fistulas with the duodenal fistula opposite the main pancreatic duct. Pancreatic juice was collected through a rubber funnel inserted by way of the duodenal fistula tube. In one dog the bile duct had been transplanted into the stomach. Since adequate drainage of the gastric contents to the outside was maintained in all of the dogs while observations were being made, in this animal only injected bile entered the intestine throughout the course of an experiment. This precaution did not, however, influence the results. Details of the methods have been described previously (Thomas and Crider, 1940; Thomas, 1941).

Two commercial preparations of desiccated ox bile were used as well as dog bile collected from the gallbladder of anesthetized animals or through the duodenal fistula from the animal under observation. The ox bile, with few exceptions, was dissolved in distilled water in whatever concentration was required to make the solution isotonic with the blood as indicated by determination of the freezing point. This concentration was 9 per cent for one preparation and 14 per cent for another. The pH of the bile preparations was generally measured and was, on occasion, adjusted to various predetermined levels in order to compare the effects of acid and alkaline bile.

The usual procedure, after preparing to record the flow of pancreatic juice with a drop recorder, was to wait until the flow had ceased or become constant and then inject 10 or 20 cc. of the bile preparation into the middle or lower duodenum or upper jejunum and record the result. In addition to experiments

devoted to the study of bile, it was used in a number of experiments designed for other purposes; as a result we have records of the action, or lack of action of bile, under a variety of experimental conditions.

Results. *Attempts to stimulate pancreatic secretion by means of bile.* Only seven of a total of sixty-two injections of bile were followed by an increase in the rate of secretion of pancreatic juice apparently caused by the bile. A certain number of "positive" results are regularly obtained following the injection of any inert substance because of the appearance at unpredictable times of spontaneous secretion from the pancreas. Most of the results classed as positive could not be repeated in the same animal on the same day. In forty of the trials there was either no change or a decrease in the rate of secretion. In fifteen of the experiments the flow increased after bile was injected but either it had begun to increase before the injection was started or the increase was too small to be

TABLE 1

Effect on pancreatic secretion of injecting bile into the intestine

DOG NO.	NUMBER OF EXPERIMENTS		
	Increase	No effect or decrease	Doubtful
3-39	5	16	5
2-39	1	14	5
2-40	1	10	5
Total...............	7	40	15
pH RANGE			
2.75-6.9	1	9	7
7.0 -8.0	4	24	6
*Not known	2	7	2
Total	7	40	15

* Mostly dog's g.b. bile, therefore below pH 7.0.

considered significant (less than 1 drop per minute) or for some definite reason it was judged to be due to other causes. These results have been classified as doubtful. They should probably be considered negative.

We saw an unmistakable increase in the flow of pancreatic juice clearly due to the injection of bile on only two occasions. The increases occurred in experiments on the same animal (3-39) on two successive days. At the time his duodenal mucosa was intensely red and congested, suggesting duodenitis. After the mucosa returned to normal this animal again gave negative results. With this exception, the results were essentially the same in all three dogs.

In view of Mellanby's suggestion that the pH of the bile determines its effectiveness as a stimulus for the pancreas, we tried bile adjusted to various pH levels but observed no difference in the effects of acid as compared to alkaline bile. Results are summarized in table 1.

We also tried mixing the bile with various foodstuffs including dextrose, dextrin, peptones, fats and soap. These experiments gave the usual proportion of negative results with regard to the action of bile. In other experiments bile was administered when the stomach contained food or had recently been emptied through the gastric fistula. Generally under these circumstances the pancreas was secreting actively but the administration of bile failed to augment the secretion.

The inhibitory action of bile. Lueth and Ivy (1927) remarked that in some of their experiments administration of bile appeared to decrease the amount of pancreatic secretion produced in response to other agents. Dragstedt and Woodbury (1934) consistently obtained less pancreatic juice from their animals when bile was administered than when it was excluded from the intestine but

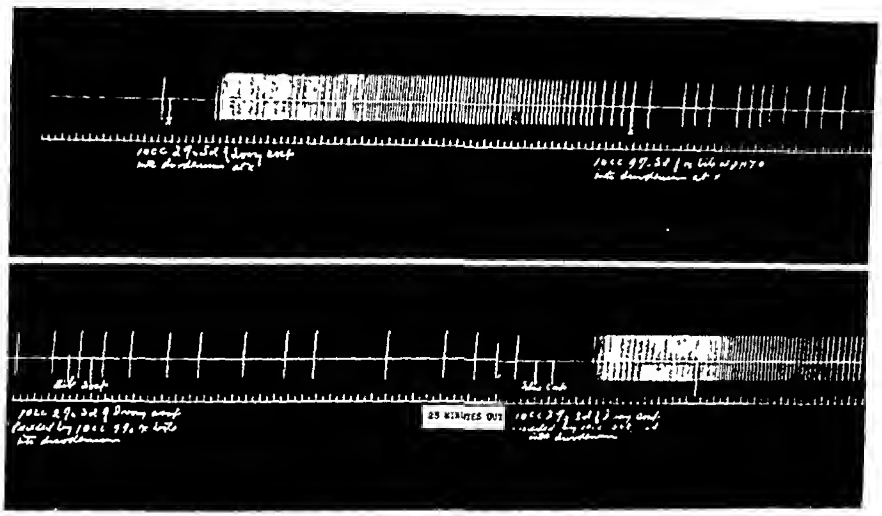

Fig. 1. Record made with a drop recorder showing the inhibitory effect of bile on the secretion of pancreatic juice due to soap (lower graph). The absence of any stimulating action of bile is also shown in the upper graph. The lower graph is a continuation of the upper one. Time is recorded in 30 second intervals.

they did not comment on the fact. In many of our experiments we have been impressed by our failure to obtain characteristic responses to other stimuli after administering bile. We, therefore, undertook a systematic study of the possible inhibitory action of bile on the response of the pancreas to other stimuli.

For this purpose two small injection tubes were passed into the intestine, one of which was between 13 and 17 inches longer than the other. Bile was injected through one (usually the longer) tube and either 5 per cent peptone solution, 2 per cent soap solution or N/20 HCl was injected through the other. With this arrangement the bile and the stimulating substance were placed in the intestine at different levels. This helped to prevent mixing and, in the case of HCl, neutralization.

In every instance, peptone, soap or HCl caused less secretion when given shortly after bile had been administered than it had previously produced when

given alone. Peptone or soap often failed to cause any secretion when bile was present and almost invariably failed if only moderate amounts of the stimulus (e.g., 10 cc.) were used. One such result is illustrated in figure 1. The results with HCl are presented in table 2.

DISCUSSION. In view of our failure to demonstrate any consistent increase in the flow of pancreatic juice following the administration of bile into the intestine of normal unanesthetized dogs, it is desirable to find the reason for Mellanby's positive results with anesthetized cats. One obvious explanation is that in a great majority of his experiments the pylorus was not tied and gastric juice, the secretion of which is stimulated by bile (J. Myer, A. C. Ivy and E. T. McEnery, 1942) was free to enter the intestine. In our experiments the gastric juice was drained to the outside through the gastric fistula. The long latent periods recorded by Mellanby and the fact that pancreatic secretion

TABLE 2

Amount of pancreatic juice secreted in response to 10 cc. N/20 HCl in the presence of bile compared to the normal response

Number of samples collected is given in parentheses beside the average volume in each instance

DOG NO.	DATE	WITHOUT BILE (AV. VOL. CC.)	WITH BILE (AV. VOL. CC.)	REMARKS
10–42	9/30/42	(3) 3.0	(4) 1.42	10 cc. N/10 HCl used as a stimulus
10–42	9/26/42	(4) 3.45	(4) 1.85	
11–42	10/12/42	(4) 2.52	(4) 0.47	10 cc. 1% $NaHCO_3$ injected into lower tube in control experiments 10/12/42
11–42	9/24/42	(5) 3.12	(5) 0.55	
2–40	4/ 7/41	(6) 10.6	(1) 5.2	Bile duct transplanted into stomach
2–40	4/ 9/41	(4) 6.66	(2) 3.4	
3–39	4/ 4/41	(1) 4.8	(1) 2.06	
3–39	4/ 8/41	(5) 6.66	(1) 4.7	

continued for hours suggest that his results were due to some such indirect mechanism. On the other hand he reported two experiments in which the pylorus and common bile duct were ligated. The results were less satisfactory in these experiments and because of the small number we cannot regard them as convincing. Nevertheless, there is reason to believe that bile may stimulate the pancreas under abnormal conditions such as frequently obtain in acute experiments (Thomas and Crider, 1941).

In contrast with the doubtful character of the evidence suggesting an excitatory effect of bile on pancreatic secretion, the evidence for an inhibitory effect on the response to various stimuli is clear and consistent. It is difficult to understand the usefulness of such an inhibitory mechanism unless it is involved in the regulation of pancreatic secretion. This is possible because most of the stimuli which increase the flow of pancreatic juice into the intestine also increase the flow of bile.

SUMMARY AND CONCLUSIONS

1. In normal unanesthetized dogs injection of ox bile or of dog bile into the intestine does not increase the rate of secretion of pancreatic juice.

2. The amount of pancreatic juice secreted in response to the presence of peptone, soap or HCl in the intestine is less when bile is also present than when it is absent from the intestine.

3. Mellanby's theory that bile is an important stimulus for pancreatic secretion is based on insufficient evidence and, in view of the contrary evidence, should be abandoned.

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OBSERVATIONS CONCERNING THE ORIGIN OF RENAL LYMPH[1]

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In a previous article (1) it was found that renal lymph contained more urea than the renal arterial and venous blood. Furthermore, it was found that the bulk of the supposedly large amount of fluid being reabsorbed by the tubules could not possibly re-enter the circulation via the renal lymphatics because of the minimal flow occurring in the latter vessels.

These findings posed two questions: 1, do the renal lymphatics drain only the larger collecting ducts of the kidney, thus accounting for the high urea content of its lymph, and 2, is renal lymph derived from the tubular reabsorbed fluid, the blood plasma or from both types of fluid.

In the present study, an attempt was made to answer the first question by a determination and comparison of the glucose content of renal and cervical lymph samples. For if renal lymph were derived from the fluid in the larger renal collecting ducts (which supposedly contains a high urea and a negligible glucose content), it would be expected that renal lymph contained a much lower glucose concentration than cervical lymph, the latter type of lymph having as much glucose as blood plasma (2).

In order to solve the second question, the inulin content of renal lymph (during the intravenous infusion of inulin) was determined and compared with that of cervical lymph for the latter is derived from blood plasma. If renal lymph is derived from, and is in diffusion equilibrium with tubular reabsorbed fluid alone, it will have little or no inulin since tubular reabsorbed fluid assumedly does not contain inulin (3); if its inulin content is equal to that of cervical lymph, it is derived from and is in diffusion equilibrium ostensibly with renal blood plasma alone; and finally, if renal lymph is in diffusion equilibrium with both the renal blood plasma and tubular reabsorbed fluid, it will have some inulin but certainly, considerably less than that in cervical lymph.

METHODS. For the determination of the glucose in renal lymph, the hilar and capsular lymphatics of the dog's kidney were cannulated according to a method previously described (1). Approximately 0.025 cc. samples of renal lymph were deproteinized according to the method of Giragossintz, Davidson and Kirk (4). After subsequent centrifugation, 0.05 cc. of the supernatant fluid was oxidized with potassium ferricyanide reagent and titrated with ceric sulfate according to the method of Giragossintz and his associates. Arterial blood (5.0 cc.) was collected at the beginning and end of the lymph cannulation. After centrifugation under oil, the plasma was tested for its glucose content exactly as described above for the glucose determination of lymph. Two renal

[1] Aided by a grant from the Dazian Foundation for Medical Research.

lymph samples were obtained from the capsular lymphatics and the remaining seven samples were obtained from the hilar lymphatics of the kidney. For control purposes, seven samples of cervical lymph were obtained at the same time that renal lymph samples were obtained, and they were analyzed for glucose exactly as described above.

For the determination of the inulin content of renal lymph, eight large dogs (18–22 kgm.) were given an infusion of 250 cc. of normal saline solution containing 20 grams of inulin beginning 30 minutes prior to lymph duct cannulation and continuing during the collection. After the lymph duct was cannulated, arterial blood (femoral) was obtained and urine was collected from a catheter inserted into the ureter of the kidney. In three of these eight dogs, lymph samples also were obtained from the left cervical lymphatic vessel at the same time that renal lymph was being collected. Two more cervical lymph samples were

TABLE 1

The glucose content of renal and cervical lymph

EXP. NO.	BLOOD PLASMA GLUCOSE (MGM./100 CC.)	RENAL LYMPH GLUCOSE (MGM./100 CC.)	CERVICAL LYMPH GLUCOSE (MGM./100 CC.)
12	135.0	115.0	
13	107.0		98.0
14	70.0	70.0	101.0
16	106.0	86.0	97.0
17	102.0	92.0	84.0
18	99.0	108.0*	107.0
19	113.0	98.0	125.0
20	99.0	90.0	91.0
21	101.0	83.0	112.0
Average..............	103.5	92.7	101.9

* Capsular lymph.

obtained from two normal dogs, receiving an inulin infusion similar to that given to the eight dogs above. After 20 minutes of renal lymph collection, a second arterial blood sample was taken and the urine collection was stopped. The inulin content of the lymph samples (renal and cervical), the two blood samples, and the urine sample was determined according to the method of Alving, Rubin and Miller (5).

Results. A. *The glucose content of renal and cervical lymph.* As can be seen in table 1, the average concentration of glucose in eight renal lymph samples was 92.7 mgm. per 100 cc. and 101.9 mgm. in the eight cervical lymph samples. This high glucose concentration in renal lymph strongly suggests that renal lymph could not be derived exclusively from the relatively sugar-free fluid contained in the larger collecting ducts of the kidney. The close similarity, however, between the glucose concentrations of renal and cervical lymph samples does not of itself indicate that renal lymph is derived exclusively from renal blood plasma, for it may be derived partially from tubular reabsorbed fluid and

still have a glucose content as high as cervical lymph, since tubular reabsorbed fluid also is supposedly high in glucose.

B. *The inulin content of renal and cervical lymph.* The average concentration of inulin in renal lymph samples taken from eight dogs receiving an intravenous infusion of inulin (and having an average blood plasma concentration of 121.2 mgm. per 100 cc.) was found to be 82.5 mgm. per 100 cc. (see table 2). It was found also that cervical lymph, collected under the same experimental conditions, contained an average inulin concentration of 138.8 mgm. as compared to an average blood plasma concentration of 147.6 mgm. Thus, unlike the similarity in the glucose values of renal and cervical lymph samples, a marked difference in the inulin content of these two types of lymph was found. For whereas cervical lymph had an inulin content 94 per cent of that in blood plasma, renal lymph had an inulin content but 68.0 per cent of that found in blood plasma. It is of interest that the average inulin clearance of the single kidney in these experiments was 57.6 cc. per minute. This large clearance indicates that these kidneys were functioning in good order despite the cannulation procedure effected upon one of their lymphatic vessels.

TABLE 2

The inulin content of renal and cervical lymph

EXP. NO.	BLOOD PLASMA INULIN (MGM./100 CC.)	RENAL LYMPH INULIN (MGM./100 CC.)	CERVICAL LYMPH INULIN (MGM./100 CC.)	INULIN CLEARANCE (CC./MIN.)	URINE VOLUME (CC./MIN.)
II	82.7	43.8*		57.4	0.84
IV	56.8	10.0*		61.8	0.67
V	194.2	142.0*		49.0	1.87
IX	108.0	81.8		36.5	1.63
X	65.9	35.0		127.0	2.20
XI	136.0		142.8		
XII	140.0		144.8		
XIII	16.0	10.8	20.0	36.9	0.98
XIV	84.0	70.0	99.5	38.3	0.97
XV	362.0	266.4	287.0	53.6	0.80
Average.....	147.6† 121.2‡	82.5	138.8	57.6	1.25

* Capsular lymph.
† Average blood level of inulin during collection of cervical lymph.
‡ Average blood level of inulin during collection of renal lymph.

Discussion. The preceding observations make it clear that renal lymph is not derived exclusively from the relatively sugar-free fluid contained in the larger collecting ducts of the kidney. For if it were, the glucose content of renal lymph would be nil or very low whereas it was found that its content of this substance was practically as high as that of cervical lymph. Thus, the high urea content of renal lymph (1) cannot be explained by the assumption that renal lymph is derived chiefly from the fluid contained in the larger collecting ducts which is high in urea.

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XIV	84.0	70.0	99.5	38.3	0.97
XV	362.0	266.4	287.0	53.6	0.80
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† Average blood level of inulin during collection of cervical lymph.

‡ Average blood level of inulin during collection of renal lymph.

DISCUSSION. The preceding observations make it clear that renal lymph is not derived exclusively from the relatively sugar-free fluid contained in the larger collecting ducts of the kidney. For if it were, the glucose content of renal lymph would be nil or very low whereas it was found that its content of this substance was practically as high as that of cervical lymph. Thus, the high urea content of renal lymph (1) cannot be explained by the assumption that renal lymph is derived chiefly from the fluid contained in the larger collecting ducts which is high in urea.

The above experiments also appear to indicate that the composition of renal lymph is determined by the character of both tubular reabsorbed fluid and the renal blood plasma. If it were derived exclusively from the renal tubular reabsorbed fluid, its inulin content would be practically nil, and if it were exclusively derived from the renal blood plasma, its inulin content would be equal to that of cervical lymph. In view of the fact that renal lymph does contain considerable inulin but at a considerably lower concentration than that found in cervical lymph, it seems reasonable to assume that it is derived from both fluid elements present in the kidney. As mentioned above, the glucose content of renal lymph, unlike the inulin content, was approximately the same as that of cervical lymph. However, this is to be expected, regardless of the source of renal lymph, since tubular reabsorbed fluid supposedly contains as much glucose as the renal blood plasma.

CONCLUSIONS

1. The glucose and inulin contents of renal and cervical lymph were determined and compared.
2. Renal lymph is not derived exclusively from the larger collecting ducts of the kidney, despite the high concentration of urea in renal lymph.
3. Renal lymph is derived apparently from both the renal blood plasma and the tubular reabsorbed fluid.

The authors wish to express their thanks to E. Lindner and Ralph Levy for their technical assistance.

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THE ABSENCE OF RENNIN FROM ADULT HUMAN GASTRIC JUICE[1]

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It is now generally accepted that pepsin and rennin are distinct and different enzymes (1, 2). However, the impression seems to be general that they are both present in human gastric juice. Most text-books make such statements and, indeed, some investigators have made similar assumptions. For example, Helmer, Fouts and Zerfas (3, 4) determined the milk-clotting power of human gastric juice and designated as "rennin" the value thus obtained, while estimating "pepsin" by another procedure. Since pepsin also clots milk, obviously their "rennin" values are really either pepsin plus rennin or pepsin alone. There is no evidence to support the assumption that rennin is present in human gastric juice.

Holter and Anderson (2, 5) studied the pepsin/rennin quotient of the gastric fluids of human subjects as well as of other species. They found that this quotient is fairly constant in the human and can not be changed by chemical influences. Their studies led them to the conclusion that rennin is produced only by the calf, and, presumably, by other ruminants.

Although Holter and Anderson's work is quite convincing, it is, nevertheless, indirect. It seemed to us that a more direct approach to the problem might be made. Tauber and Kleiner (6) showed that pepsin solutions rather rapidly digest rennin. This seemed to afford a means of detecting rennin in gastric juice. If the clotting power of gastric juice, determined soon after it is collected, suffers considerable reduction on incubation, it may be considered evidence of the digestion of the rennin by the pepsin-HCl. On the other hand, if no diminution of power takes place in gastric juice containing active pepsin it would show that no rennin is present.

The technique used to measure the clotting-power was the Barowsky-Tauber-Kleiner clinical method of estimating pepsin (7). Its basis is the determination of the smallest quantity of diluted gastric juice that will just clot 10 cc. of milk, buffered to pH 5.0 in ten minutes.

Gastric fluid was obtained from adult subjects following a "test meal" of 150 cc. of 7 per cent ethyl alcohol or a histamine injection. If the fluid was not acid, it was acidified to congo red. The clotting power was immediately determined. The sample was then incubated at 38°C. for 24 hours, after which the clotting power was again measured.

The table (table 1) gives the results of 15 experiments. In one case (no. 6) there is a slight increase in activity, possibly indicating a completed conversion

[1] A preliminary report of this work was presented before the American Physiological Society at Boston, Mass., April 3, 1942; Federation Proceedings 1: 21, 1942.

of pepsinogen to pepsin. In two experiments (nos. 11 and 13) a slight decrease might be interpreted as due to a trace of rennin. However, they represent a difference of 0.1 and 0.2 cc. of gastric juice added, respectively.[2] We are inclined to regard them as errors due to the method. All the other twelve experiments show no change in clotting power.

To check the remote possibility that the entire clotting power was due to rennin rather than pepsin, a 1:1 mixture of the gastric fluid and pepsin solution (containing 833 units/cc.) was incubated for 24 hours. This was done in five experiments (nos. 6–10). The differences between the amounts found and those calculated, assuming no rennin was present, are inconsequential.

TABLE 1

SAMPLE NUMBER	GASTRIC FLUID AS COLLECTED (PEPTIC UNITS PER CC.)	GASTRIC FLUID AFTER INCUBATION AT 38°C. FOR 24 HOURS (PEPTIC UNITS PER CC.)	10 CC. OF GASTRIC FLUID PLUS 10 CC. OF RENNIN SOLUTION (833 UNITS/CC.) AFTER INCUBATION AT 38°C. FOR 24 HOURS		10 CC. OF GASTRIC FLUID PLUS 10 CC. OF PEPSIN SOLUTION (833 UNITS/CC.) AFTER INCUBATION AT 38°C. FOR 24 HOURS	
			Peptic units found	Peptic units calculated assuming all rennin destroyed	Peptic units found	Peptic units calculated assuming no rennin present
1	500	500				
2	667	667				
3	625	625				
4	625	625	312	312		
5	357	357	180	180		
6	714	1250	625	625	833	1041
7	1250	1250	625	625	1000	1041
8	5000	5000	2500	2500	3333	2917
9	1667	1667	834	834	1250	1250
10	2500	2500	1250	1250	1666	1666
11	1000	833	417	417		
12	833	833	500	417		
13	625	500	417	313		
14	714	714	417	357		
15	313	313	179	157		

In order to be sure that the conditions were suitable for digestion of rennin, a 1:1 mixture of gastric fluid and rennin solution (833 units/cc.) was likewise incubated. In nearly all the 12 experiments in which this was tried all the rennin was digested in 24 hours.

We have other records of the same general tenor. In some instances human gastric fluid remained at refrigerator temperature for days and even weeks with no change in clotting power. Corroboration of these results has been obtained

[2] The empirical formula for calculating the unitage is Units/cc. = (1/volume of diluted gastric fluid × 10) × degree of dilution. The dilutions are (1) undiluted, (2) 1:25 or 1:50 and the volumes used are 0.1 to 0.9 cc. in 0.1 cc. increments. Thus, a change of 0.1 cc. gives a fairly large variation in the total number of units found. A clinical unit is extremely small being equivalent to 0.04 gamma pepsin nitrogen. This value was determined by using a solution of crystalline pepsin for which we wish to thank Dr. J. H. Northrop.

with samples from normal and pathological hospital cases (8). The fact that all clotting power of human gastric juice is due to pepsin alone would explain the fact that pepsin and "rennin" seem to run parallel in normal and pathological cases, according to Helmer, Fouts and Zerfas' observations.

SUMMARY

By using the phenomenon of the digestibility of rennin by pepsin as a basis for the detection of rennin, it was found that no rennin was present in the gastric juice of human adults.

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HISTOLOGICAL STUDIES OF THE PANCREAS AND ASSOCIATE TISSUES OF WILD AND EXPERIMENTALLY FED YOUNG CHINOOK SALMON

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The general results of two feeding experiments using chinook salmon finger lings (*Oncorhynchus tschawytscha*) as test animals were described by Donaldso and Foster (1939) and Norris and Donaldson (1940). The pancreatic-intestina region of representative fish of each lot in the experiments mentioned was pre served at the end of the experiment and subjected to a histological study. Th results of this study indicate very pronounced changes may take place in th pancreas of chinook salmon fingerlings as a result of various diets.

Gaschott (1931), Hayford and Davis (1936), and Hewitt (1937a and 1937b described diseased conditions in trout livers produced by faulty diet. Hes (1935) found that profound changes occurred in the pancreas of fish under dif ferent dietary and environmental conditions.

The studies of Hess, of Dragstedt et al. (1936) and of Van Prohaska et al (1936) on liver changes in depancreatized dogs suggested that the pancreati changes might be closely linked with fatty degeneration of the liver and might, in fact, precede the liver changes. The studies on chinook salmon carried on to date seem to indicate that such is the case.

Discussion of the histological condition of wild and experimentally fed fish. A study of normal pyloric ceca-pancreatic tissue preceded the study of the mate- rial from the fish of the experimental lots. Chinook salmon were obtained from wild stocks in areas where it is almost certain they had not received other than natural food substances. Representative tissue from a wild fish, with standard length of 53 mm., is presented in figure 1.

The pancreatic tissue of these wild fish has very dense acinar tissue, the cells of which are distinct. Fat is interspersed among the ceca, or in some cases infiltrates into the acinar tissues of the pancreas. The tissues from fish with lengths of 49, 53, 87 and 102 mm. show very definite fat deposits in the pancreatic tissue. The tissues from a 32-mm. fish, a fish just beginning to feed, show very little fat infiltration in the pancreatic tissues but a generous amount of fat tissue is located between the loops of the intestine. A single fish, one 69 mm. in length, failed to reveal fat tissue deposited either among the ceca or in the pancreatic tissue.

The tissues from the region of the pyloric ceca and intestine of the fish studied are similar to the tissues described by Greene (1914, 1915) from young chinook salmon, and Barr (1935) from the adults.

The cross sections of the intestine, pyloric ceca, and pancreas of the fish fed experimentally show some with tissues very similar to those of the wild fish, and others with marked changes in the tissues.

The cross sections of the liver-fed fish, lot 1, reveal tissues very similar to those figured and described by Hess (1935). Considerable fatty infiltration of the pancreas has taken place. The acinar cells that remain, however, appear to be normal. Islets of Langerhans occur scattered throughout the tissue. The infiltration of the fat in this case was not reflected in any adverse condition noted externally in the fish. The rate of growth and low mortality at the termination of the experiment lead one to conclude the fat infiltration in this case was not excessive in amount.

Cross sections of pancreatic tissue in the fish fed a diet of salmon viscera alone show very marked differences from those of fish that were fed an exclusive beef liver diet. The amount of fatty infiltration was much less in the pancreas of the fish fed salmon viscera. The acinar tissue has a very indistinct appearance, with some cell material evidently breaking from the cells and lodging in the spaces between the pyloric ceca and the pancreatic tissue. This condition is indicative of the initial stages of the breakdown of the tissue. The mortality rates and rate of growth of the fish in this experimental lot were such that one might assume the condition was not, as yet, extremely injurious to the fish.

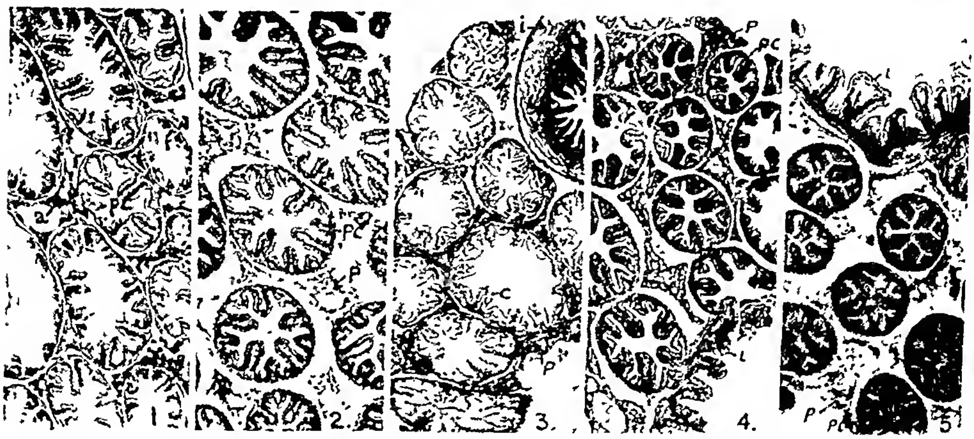
The fish in lot 3, fed a diet of 40 per cent beef liver and 60 per cent seal meal, present a condition slightly more aggravated than the fish in lot 2. The pancreatic tissue in these fish appeared to be in the initial stages of degeneration, with some breakdown in the cells. The mortality increased during the last two weeks of the experiment,—a further indication of an incipient dietary deficiency.

The fish fed diet 5, composed of 20 per cent beef liver, 20 per cent salmon viscera and 60 per cent seal meal show, both macroscopically and microscopically, all the symptoms of the diseased condition usually referred to as fatty degeneration of the liver. In addition these fish contained pancreatic tissue in the advanced stage of degeneration (fig. 2). The actual pancreatic tissue had largely disappeared leaving only cell fragments, connective tissue, and fat cells between the pyloric ceca. A marked increase in the mortality rate further indicated that the diet was inadequate for the young fish.

The fish that were fed a diet of one-third beef liver, one-third salmon viscera, and one-third seal meal developed pancreatic tissues that were apparently normal. The tissues of these fish, with an average length of 63.7 mm., are very similar to those of wild fish of about the same length.

Fish that received 20 per cent ground beef pancreas, 20 per cent beef liver, and 60 per cent seal meal as their diet, lot 7, developed pancreatic tissue which appeared very similar to that produced by fish fed a diet of 100 per cent beef liver. The large amount of fatty tissue in the pancreas of the fish in this experimental lot may have been produced by the large amounts of beef fat that were mixed with the beef pancreatic tissues. The very remarkable differences in the tissues of the fish fed diets 3, 5 and 7, demonstrate clearly a pronounced effect produced by dietary changes. The replacing of 20 per cent of the beef liver of diet 3 with the 20 per cent beef pancreas in diet 7, resulted in a great increase in the fatty tissue in the fish pancreas. The acinar tissue of the fish

fed diet 7 appeared to be distinct and normal. The differences in the pancreatic tissue of the fish fed diet 7 with 20 per cent beef pancreas, as compared with the fish that received diet 5, which contained 20 per cent salmon viscera, would lead one to conclude that the addition of beef pancreas to the diet had a very



The photomicrographs of the cross sections through the region of the pyloric ceca, pancreas, and intestine were made with uniform magnification (× 30). The variation in size of the pyloric ceca is due largely to the variation in size of the fish from which the materials were removed.

Abbreviations: *a*—acinar tissue of the pancreas; *c*—columnar epithelium; *f*—fat tissue; *i*—intestine; *p*—pancreas; *p.c.*—pyloric ceca.

Fig. 1. Photomicrograph of the cross section of the pancreas, pyloric ceca, and intestine of a 53 mm.-chinook salmon fingerling captured in the San Joaquin River, California.

Fig. 2. Photomicrograph of the cross section of the pancreas and pyloric ceca of a chinook salmon fingerling from a group averaging 64.6 mm. in length that had been fed diet 5 (20 per cent beef liver, 20 per cent salmon viscera, 60 per cent seal meal) for twenty weeks. The pancreatic tissue shows almost complete degeneration.

Fig. 3. Photomicrograph of a cross section of the pancreas, pyloric ceca, and intestine of a chinook salmon fingerling from a group averaging 42.6 mm. in length that had been fed diet 11 (basic diet + 20 parts of salmon oil, 2 parts of cholesterol) for sixteen weeks. The pancreatic tissue shows some degeneration. The columnar epithelium of the pyloric ceca is greatly thickened and vacuolated.

Fig. 4. Photomicrograph of a cross section of the pancreas, pyloric ceca, and intestine of a chinook salmon fingerling from a group averaging 49.3 mm. in length that had been fed diet 12 (basic diet + 20 parts of starch, pancreas extract) for sixteen weeks. The pancreatic tissues are apparently normal.

Fig. 5. Photomicrograph of a cross section of the pancreas, pyloric ceca, and intestine of a chinook salmon fingerling from a group averaging 50.2 mm. in length that had been fed diet 13 (basic diet + 20 parts of salmon oil, 2 parts of cholesterol, pancreas extract) for sixteen weeks. The pancreatic tissue is almost completely disintegrated.

beneficial effect, while the salmon viscera in a similar diet had a very injurious effect on the pancreatic tissues of the young chinook salmon.

Twenty per cent apple flour combined with 40 per cent seal meal and 40 per cent beef liver, and fed to fish in lot 8, resulted in pancreatic tissue very similar to that of fish fed diet 3. The supplementing of part of the seal meal with 20 per cent apple flour did not produce a noticeable difference in the pancreatic tissues.

Pancreatic changes in fish fed synthetic diets. The growth and mortality rates of the fish fed on synthetic diets (nos. 9–14) were discussed in a previous report (Norris and Donaldson, 1940).

The fish fed diet 9, in which 80 parts of the basic diet[1] were supplemented by the addition of 20 parts of starch, showed very little structural modification of the tissues.

Pancreatic tissues of the fish fed diet 10, in which 80 parts of the basic diet were supplemented with 20 parts of salmon oil, show very pronounced degeneration.

The tissues of the fish fed diet 11, in which 80 parts of the basic diet were supplemented with 20 parts of salmon oil and 2 parts of cholesterol (fig. 3), show most extraordinary modification. The cells of the columnar epithelium of the pyloric ceca and parts of the intestine are greatly enlarged. The condition of these cells is similar, although much more pronounced than that of tissues from the same region of young chinook salmon, figured by Greene (1915), that were fed olive oil by rectal injection.

The addition of pancreatic extract and 20 parts of starch to the basic diet (12) produced pancreatic tissues that appeared to be very nearly normal. The photomicrograph of tissues from representative fish from this lot (fig. 4), of an average length of 49.3 mm. is very similar to those of wild fish of 49 mm. length. The differences in the tissues of the fish fed diet 12 and those fed diet 9 may be attributed to the adding of the pancreatic extract.

The beneficial effect of feeding the pancreatic extracts in this case is similar to the results obtained by Van Prohaska et al. (1936) and Dragstedt et al. (1936), who found that the "fat-free alcohol extract" of beef pancreas contained an active principle that was effective in preventing fatty liver in depancreatized dogs. To this active substance they gave the name "lipocaic" and described it as a fat metabolizing hormone.

Figure 5 represents a photomicrograph of the cross section of fish fed diet 13. This diet consisted of the basic diet supplemented with 20 parts of salmon oil, 2 parts of cholesterol, and pancreatic extract. The pancreatic tissues show almost complete disintegration while the columnar epithelium cells of the pyloric ceca appear normal. The differences between the tissues photographed in figure 5 and those in figure 3 can be said to be due to the addition of pancreatic extract in the former case. The differences between the fish tissues reproduced in figures 5 and 4 can be attributed to the addition of salmon oil and cholesterol in the diet of the former, to replace the starch in the latter diet.

The addition of choline-hydrochloride to diet 14, which in addition to the basic diet contained 20 parts of salmon oil and 2 parts of cholesterol, seemed to have some beneficial effect in preventing abnormal changes in the tissues. This result parallels the findings of Halliday (1938) who found that the addition of choline to the diets of rats suffering from fatty liver exerted a remedial effect.

It might be concluded that the addition of 20 parts of salmon oil, or salmon oil and 2 parts of cholesterol, to the basic diet produced destructive changes in

[1] The basic diet used was composed of the following parts: meat 55, salt 5, yeast 8, cod liver oil 2, gelatin 10, and liver extract.

the pancreatic tissue. The addition of pancreatic extract of choline to the diets exerted a beneficial effect in preventing these changes.

SUMMARY AND CONCLUSIONS

Representative fish from experimental lots of fish fed various diets were preserved, and the pyloric ceca-pancreatic region studied by cross sectioning the area and comparing it with tissues from the same region from wild fish.

These studies indicate that profound changes occur in the pancreatic tissue of fish as a result of the diet.

The fish fed a diet of 100 per cent beef liver had excessive fat deposited in the pancreatic tissues.

Diets of 20 per cent salmon viscera, 20 per cent beef liver, and 60 per cent seal meal induced degeneration of the pancreatic tissue, while fish fed a diet of $\frac{1}{3}$ salmon viscera, $\frac{1}{3}$ beef liver, and $\frac{1}{3}$ seal meal had normal appearing pancreatic tissue.

Fresh beef pancreas in the diet added to the fat content of the fish pancreas, but in other respects the fish pancreatic tissues appeared normal.

The addition of salmon oil, or salmon oil and cholesterol to synthetic diets produced destructive changes in the fish pancreatic tissues. The addition of pancreatic extract or choline to the synthetic diets produced beneficial effects in preventing the destructive changes.

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THE EFFECT OF SYMPATHOMIMETIC AMINES UPON THE OUTPUT OF RESPIRATORY TRACT FLUID IN RABBITS

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Perry and Boyd (1) have reported that stimulation of the cervical vagus nerve and injections of pilocarpine nitrate will produce a marked increase in the output of respiratory tract fluid (R.T.F.) collected from a cannula in the trachea of urethanized rabbits and cats. In the present communication corresponding data will be presented upon the effect of faradic electrical stimulation of the cervical sympathetic trunk and upon the effect of adrenaline hydrochloride and other sympathomimetic amines.

Most of the work was performed upon healthy, adult rabbits with a few experiments upon guinea pigs. The animals were anesthetized with urethane and a side arm cannula ligated into the trachea after the method of Perry and Boyd (1). Subsequently, several modifications were made from the method of Perry and Boyd. The side arm of the tracheal cannula was connected with a heat-insulated, double right angle bent glass tube which, in turn, connected with a glass tube of wide bore housed in a box wherein the temperature was kept constant at 38°C. by an electric bulb connected in series with a De Khotinsky thermoregulator and condenser and a small electric fan to maintain a circulation of air. The wide bore glass tube passed directly through this box and inside the wide bore tube were plugs of cotton lying upon a wire tray and kept continuously moist by water, dripping at a rate regulated by a Hoffmann screw clamp upon a piece of rubber tubing which connected to a reservoir Mariotte bottle of water placed on the top of the heating box. This arrangement substituted for the nasal passage, which had been eliminated, and warmed and moistened the air which the rabbits inhaled. R.T.F. which drained from the trachea was collected in a graduated, heat-insulated, 15 ml. centrifuge tube attached to the distal end of the tracheal cannula.

The animals were fastened upon their backs or bellies to especially built operating tables in which the copper-covered, heated top could be raised from a base upon a hinge located at the head of the table and thus hold the animal, head downward, at any satisfactory angle which permitted a slight decline in the tube which received the R.T.F. The actual posture of the body has been

shown by Boyd and Ronan (2) to be without influence upon the volume of R.T.F. collected.

Thus assembled, R.T.F. was collected over a period of 3 hours. The volume of R.T.F. was noted at intervals of one hour, divided by the weight of the animal in kilograms and multiplied by 24 to give a figure which indicated the output of R.T.F. in milliliters per kilogram body weight per 24 hours. During the first hour the output was erratic but by the end of the third hour it had settled down to a fairly steady rate in most animals. At the end of the third hour adrenaline hydrochloride, dissolved in the B.P. vehicle, was injected subcutaneously in various doses and in a volume of 1 ml. per kilogram body weight. Controls received at the same time an equivalent volume of adrenaline vehicle only. A summary of the results has been given in table 1 in which the output

TABLE 1

The effect of various sympathomimetic drugs upon the output of respiratory tract fluid

ANIMAL	NO. OF ANIMALS	DRUG	DOSE (MGM./KILO)	OUTPUT OF R.T.F.-ML./KILO/24 HRS.					
				Hrs. before drug		Hrs. after drug			
				2	1	1	2	3	4
Guinea pig	13	Controls		1.7	1.8	1.6	2.2	2.1	2.0
Guinea pig	11	Adrenaline	0.1 to 1	2.5	2.9	4.3	2.7	2.8	2.8
Rabbit	18	Controls		3.3	3.4	3.8	3.0	3.2	3.4
Rabbit	7	Adrenaline	1	1.7	2.5	2.0	3.3	2.6	2.5
Rabbit	13	Adrenaline	5	2.7	2.7	2.8	2.2	3.2	1.7
Rabbit	14	Ephedrine	10 to 500	3.1	4.1	4.2	3.5	3.3	3.4
Rabbit	8	Neo-synephrine	10 to 500	2.8	4.4	4.5	3.4	2.8	2.4
Rabbit	8	Amphetamine	1	2.2	2.2	2.4	2.3	1.9	1.5
Rabbit	12	Amphetamine	10	3.5	4.1	4.5	5.5	4.7	4.4
Rabbit	9	Privine	1	2.6	3.0	4.1	3.9	1.9	1.9
Rabbit	19	Privine	10	3.1	3.3	4.1	3.8	3.5	2.3
Rabbit	14	Sympathetic stimulation		2.9	2.6	3.2	2.1	2.5	2.1

of R.T.F. has been given for the two hours immediately preceding the injection of adrenaline hydrochloride or vehicle and for four hours after.

Adrenaline hydrochloride was given in doses of 1 and 5 mgm. per kilogram body weight. As shown in table 1, the injection of this drug had no effect upon the output of R.T.F. A few doses of 10 mgm. per kilogram were tried but proved lethal in most rabbits. Corresponding doses given to guinea pigs gave an increase in the mean output one hour after injection which was due to two exceptionally high values; statistical calculations failed to reveal any significant effect of the drug.

Ephedrine hydrochloride was injected in a range of doses from 10 to 500 mgm. per kilogram body weight. As with adrenaline hydrochloride, there were no significant changes in the output of R.T.F.

Neosynephrine hydrochloride, N.N.R., was also injected, in doses similar

to those of ephedrine hydrochloride. Likewise it had no effect upon the amount of R.T.F. drained from the trachea of rabbits.

Amphetamine (Benzedrine) sulphate, N.N.R., was injected subcutaneously, in doses of 1 and 10 mgm. per kilogram body weight. Larger doses of 50 mgm. and over were tried but proved lethal to most rabbits. The dose of 1 mgm. per kilogram had no effect upon the output of R.T.F. Following the injection of 10 mgm. per kilogram, amphetamine increased the mean output of R.T.F. over a period of several hours. The results suggested that amphetamine might have some small effect upon the volume of R.T.F. excreted, an effect which, in view of the results with the previous sympathomimetic amines, was probably related to the stimulant effect of amphetamine upon the central nervous system rather than to its local sympathomimetic action which latter, in any event, is considerably different from that of adrenaline (3).

Another sympathomimetic substance investigated was Privine or 2-(naphthyl-1-methyl) imidazoline hydrochloride, a new derivative of histamine with an adrenaline-like action upon arterial muscle (4). It was injected in doses of 1 and 10 mgm. per kilogram body weight. There was some increase in the mean output of R.T.F. following the administration of this drug, but this was slight and not statistically significant.

Thus none of these sympathomimetic drugs, with the possible exception of amphetamine, had any significant effect upon the volume output of R.T.F. A corresponding result was obtained by faradic electrical stimulation of the cervical sympathetic nerve trunk.

The cervical sympathetic trunk lying in the carotid sheath was dissected carefully from the vagus nerve and the carotid artery for a length of about 1 cm. with the rabbit under urethane anesthesia. The nerve was raised slightly from nearby tissues from which it was then separated by a thin sheet of condom rubber and from time to time the nerve was moistened with saline solutions. Silver electrodes were applied to the nerve which was stimulated every 5 seconds for a period of 1 second with a faradic electrical current from an induction coil connected in series with a Brodie cut-out key. From the point of stimulation, afferent fibres pass downward to the inferior cervical ganglion from which arise the efferent fibres to the lung. While it could not be stated with certainty that impulses actually reached the acinar glands of the bronchial mucosa by stimulation of the sympathetic at the point described, nevertheless this technique was considered preferable to stimulation at or below the inferior cervical ganglion which involved artificial respiration, an undesirable complication in experiments designed to measure the output of R.T.F. Thus assembled, R.T.F. was collected from rabbits over a period of 2 to 3 hours, until a steady rate had been obtained. Then the cervical sympathetic nerve was stimulated for a period of from 1 to 3 hours and its effect upon the output of R.T.F. noted. After this followed a period of no nerve stimulation for a corresponding length of time and these two procedures were repeated, as time permitted, and alternated in different animals.

A total of 18 such experiments was performed. The mean output of R.T.F.

during sympathetic nerve stimulation was 3.5 ml. per kilogram body weight per 24 hours and the mean output during the periods of no sympathetic stimulation was 3.3 ml. per kilogram body weight per 24 hours.

These results indicated that stimulation of the cervical sympathetic nerve by this method had no effect upon the output of R.T.F. It seemed desirable to find if continuous stimulation over a longer period of time might be with effect. Consequently, rabbits were arranged as before and the cervical sympathetic trunk stimulated at 5 second intervals over a period of up to 12 hours. Controls were treated in a similar manner but given no faradic stimulation. There were 12 control rabbits and 14 rabbits with electrical stimulation of the cervical sympathetic trunk. The mean hourly output of R.T.F. in each group was calculated and plotted and the two curves were almost identical. The mean output of 75 hourly readings upon the controls was within 4 per cent of the mean output of 98 readings upon the cervical stimulated rabbits. For comparison with the output in rabbits receiving the sympathomimetic drugs, the means over corresponding hours have been included in table 1. These data indicated that stimulation of the cervical sympathetic trunk at the point indicated and with a faradic electrical current had no effect upon the output of R.T.F. These results are in line with those obtained from the administration of sympathomimetic drugs.

SUMMARY

The subcutaneous injection of a range of doses of the sympathomimetic drugs adrenaline, ephedrine, neosynephrine, amphetamine and privine was without effect upon the volume output of respiratory tract fluid drained from the trachea of urethanized rabbits, with the possible exception of large doses of amphetamine which may have slightly increased the output. Similarly, faradic electrical stimulation of the cervical sympathetic trunk had no effect upon the output of R.T.F.

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PLASMA PROTEINS (ALBUMIN AND GLOBULIN) AND RED CELL VOLUME FOLLOWING A SINGLE SEVERE NON-FATAL HEMORRHAGE

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In a previous communication (1) in this Journal, observations were reported on the changes in the plasma proteins and in the red cell volume following a single severe hemorrhage from which the dogs recovered. Immediately after the bleeding the blood was replaced by the same volume of Ringer's solution, most of which rapidly left the circulation, a level being reached in about one hour. The observations were continued for only 6 hours thereafter, and showed that in this second period the hypoproteinemia produced was only slightly affected, but that there was evidence of an increase in the plasma volume and in the total plasma proteins.

In the present communication these observations are carried further, changes being observed in a larger series of experiments for 24 hours after the hemorrhage and in a smaller series of experiments up to 7 days after the hemorrhage, both with and without replacement.

Previous work. Earlier observations on the effect of severe hemorrhage on the plasma proteins were described in the previous paper (1). Since then a number of additional studies have been made. Ebert, Stead and Gibson (2) studied 6 human donors following a bleeding of 760 to 1220 cc. After the initial fall they found an increase of plasma volume (determined by the dye method) approximately equal to the volume of red cells removed. The concentration of plasma protein (determined by calculation from the specific gravity as measured by the falling drop method) fell and then rose only slightly for several days. Notable was the observation that the hematocrit value fell for 70 hours and paralleled the increase in plasma volume. In contrast to these findings are those of Wallace and Sharpey-Shaffer (3) who also studied donors after a loss of 500 to 1150 cc. of blood. Plasma protein (determined by Nesslerization) showed little change in practically all of the 28 cases; even the hemoglobin showed little change but did fall, reaching a maximum between 3 and 90 hours with an average of 32 hours.

In dogs, Calvin (4) bled 25 per cent of the calculated blood volume without fatality and studied the changes in plasma volume, albumin and globulin for 4 hours. Proteins were determined by a Kjeldahl method. The plasma volume rose due to the inflow of fluid containing protein which was assumed to be primarily albumin, inasmuch as the albumin-globulin ratio increased. Ebert, Stead, Warren and Watts, also in dogs (5) studied the spontaneous plasma protein replacement following moderate (2 to 3.5 per cent of body weight) as con-

trasted with much more severe (repeated) hemorrhage, and found that the process was impaired in the latter group. Fine, Fischman and Frank (6) also studied the effects of severe hemorrhage in dogs both with and without anesthesia; plasma proteins were determined by calculation from the specific gravity as measured by the falling drop method. Only the change at 4 hours after hemorrhage was measured and a variable but incomplete return of the total circulating protein was observed on experiments in which no parenteral fluid was given. Price, Hanlon, Longmier and Metcalf (7) bled 15 dogs under nembutal anesthesia a total of about 3.4 per cent of their body weights, all animals succumbing apparently in about 24 hours. They found that the change in the total circulating plasma protein could be accounted for by the amount removed. However, the concentration did fall slightly due presumably to a shift of protein-free fluid into the blood stream. Plasma volume fell and there was therefore little change in the hematocrit. Plasma proteins were calculated from the specific gravity of the plasma by the falling drop technic. Brandhendler et al. (8), in cats, found a fall in serum protein as high as 79 per cent in a series of severe hemorrhages, the return to normal requiring 6 days. In horses they found that 10 days were required for the correction of the hypoproteinemia following a severe bleeding. In 7 rabbits, Volinets (9) found after a hemorrhage of 25 per cent of the estimated blood volume that the albumin and globulin following bleeding returned toward normal within 24 hours and were complete in 10 days, though the albumin returned faster than the globulin.

Experimental procedures. Three groups of experiments were carried out. In the first group of 25 dogs, the standard hemorrhage as previously described (1) was carried out. Bleeding was conducted under local anesthesia by exposing the femoral artery which was cannulated and 35 cc. per kilogram of body weight of blood removed. Immediately the same volume of Ringer's solution was replaced through the same cannula.

In the second group, 12 dogs were bled the same amount and observed for 7 days. In 6 of these experiments, Ringer's solution was used as replacement, whereas in the remaining 6 a red cell suspension in Ringer's solution was used, constituting therefore a single plasmapheresis.

In the third group of 8 dogs, no fluid at all was replaced. The amount of blood removed was somewhat larger; in half it was 40, in the others 45 cc. per kilogram of body weight. All recovered.

The entire bleeding procedure rarely required more than a few minutes. Although a fall of blood pressure and moderate shock did occur, there were no fatalities with the exception of 2 animals in the last group in which no fluid was replaced. These experiments were discarded. All observations, therefore, were made on animals who recovered spontaneously from the experimental procedure. Previous to bleeding, no food had been ingested for 24 hours. After the hemorrhage, water was allowed ad libitum for 24 hours, after which a normal kennel ration was provided.

Samples of blood were obtained from the jugular vein, heparinized and immediately centrifuged at 3000 R.P.M. at 30 minutes to determine the red cell

volume; the supernatant plasma was then analyzed for total nitrogen and fractionated for albumin and globulin after subtraction of the non-protein nitrogen, which was determined with Nessler's reagent. For nitrogen determinations the titrimetric micro-Kjeldahl procedure described by Sobel, Yuska and Cohen (10) was used. Fractionation was carried out by the method described by Campbell and Hanna (11).

Experimental findings. In all experiments the results were calculated as a percentage change, using the initial value as 100. In the first group of 25 dogs, observations were made at 1 hour, 7 hours and 24 hours after the hemorrhage and plotted as a scatter diagram in figures 1 and 2, each point representing

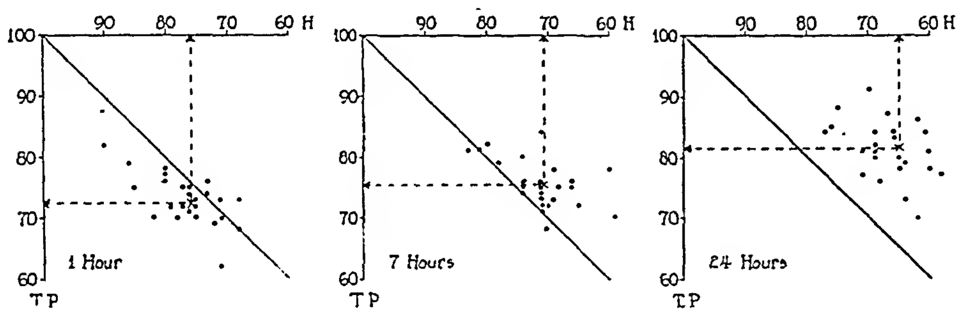

Fig. 1. Relation between red cell volume and concentration of total protein. Scatter diagram of 25 bleeding experiments described as group I in the text; each point represents one experiment. The average initial value shown as 100 per cent was, for the total protein (T.P.), 6.28 grams per cent, for the hematocrit (H) 48.8 per cent. The 45° line represents the theoretical distribution if hemodilution occurred with a protein free fluid, i.e., the red cells and the total protein would be affected equally. The crosses and arrows represent the arithmetic means and make it easier to follow the trend between 1, 7 and 24 hours.

Note the progressive fall in the average hematocrit value, indicating increasing hemodilution, most marked at 1 hour. The greater fall in protein as compared with hematocrit at 1 hour probably indicates the addition of red cells to the diluting fluid during this period. Note also (after the fall at 1 hr.) the increase of the total protein at 7 hours and 24 hours, indicating that a protein-containing fluid was responsible for hemodilution during these latter periods.

one experiment. In figure 1, the relationship between the total protein and the hematocrit value is shown. It is clear from a study of this figure that the red cell volume falls rapidly one hour after the hemorrhage and continues during the entire period of observation. This is also true of the concentration of the total protein during the first hour. However, after the first hour the fall in the total protein was replaced by a rise at 7 hours, which is continued at 24 hours. In figure 2, the relationship between the albumin and globulin indicates that the fall in globulin is slightly less pronounced than albumin at the first hour but that the increase in the concentration of total protein at 7 hours is due almost entirely to an increase in albumin fraction, confirming the findings of Calvin (4) as already mentioned. At 24 hours the changes in albumin and globulin are about the same. These changes are also shown in figure 4, which represents the mean of these values in the form of a graph.

In the second series of 12 experiments represented in figure 3, a picture is obtained of the changes occurring during one week after hemorrhage. It is obvious that the fall in the red cell volume continues for 72 hours, after which it is replaced by a slight rise at 7 days. The albumin fraction on the contrary, as shown also in figure 2, begins to increase after the precipitate fall at 1 hour; this increase is slowed at 72 hours and even at 7 days, the original concentration of albumin falls short of being reached by about 10 per cent. In contrast is the rapid return of the globulin fraction which reaches its normal level between the 24 and 72 hour specimens, continuing its increase so that at one week it has exceeded the original concentration by about 15 per cent.

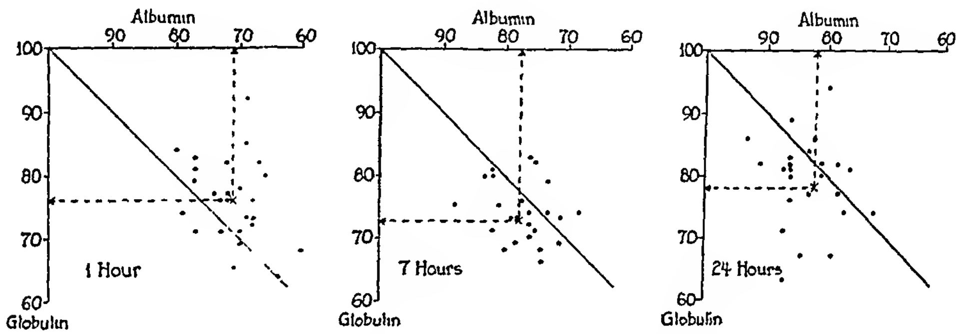

Fig. 2. Relation between concentration of albumin and globulin. Seatter diagram of the 25 bleeding experiments described in the text as group I. Eaeh point represents one experiment. The average initial value represented as 100 per eent was, for the globulin, 2.5 grams per eent, for the albumin 3.68 grams per eent. The 45° line represents the theoretical distribution if the albumin and globulin behaved the same during the hemodilution. The erosses and arrows represent the arithmetie means and make it easier to follow the trend between 1, 7 and 24 hours; these means together with those of hematocrits are plotted as a curve in figure 4.

Note the slightly greater fall in albumin than globulin at 1 hour, indicating the addition of more globulin (fibrinogen?) than albumin to the diluting fluid at this time. At 7 hours, however, note the pronounced shift indicating the addition of relatively more albumin than globulin as shown by the increased concentration of this fraction in contrast to a further fall in the globulin. At 24 hours, however, the change in the albumin and globulin fractions is about the same, indicating a shift in the opposite direction, which, as shown in figure 3 and discussed in the text, is the beginning of the globulin regeneration which far outstrips the albumin from this point on.

Of special interest are the findings in the 6 experiments of group 2, in which plasmapheresis was carried out. As will be noted by consulting figure 3, the 1 hour specimen, of course, showed no fall in hematocrit, inasmuch as approximately the same volume of red cells was replaced as was removed. Nevertheless, in 24 hours the hematocrit value dropped more rapidly than in the animals in which the replacement consisted entirely of Ringer's solution. From this point on the curve of red cell volume parallels exactly the curve in the latter experiments. The steeper fall in the red cell volume between the first and 24 hour period is obviously due to a greater hemodilution and undoubtedly explains the difference in the behavior of the albumin and globulin fractions during

this period as compared with the bleeding in which Ringer's solution alone was replaced. Thus the albumin fell after plasmapheresis, in contrast to the rise in the experiments in which Ringer's solution alone was replaced. The change in the globulin showed a similar effect, i.e., its rise was less pronounced than in the animals in which Ringer's solution was replaced. After the 24 hour sample,

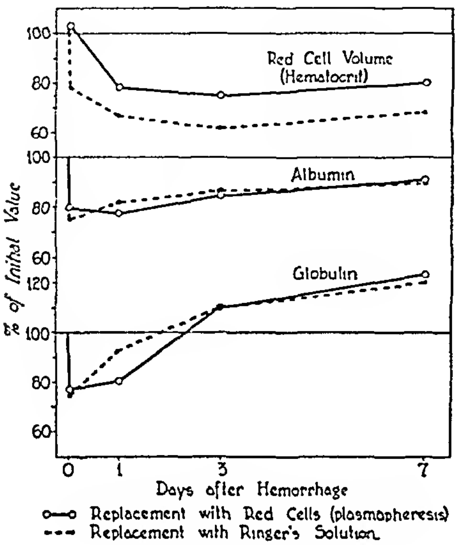

Fig. 3

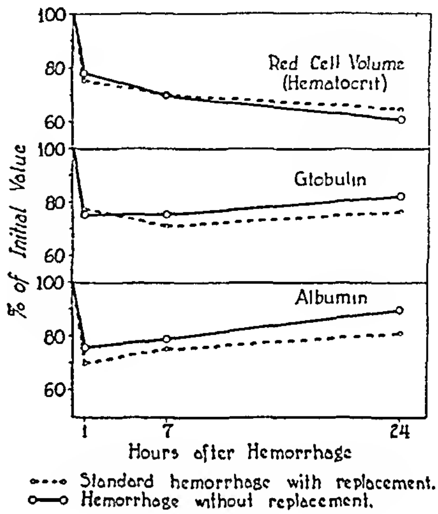

Fig. 4

Fig. 3. Relationships during one week. The two series of curves represented above were each plotted from the average values obtained in 6 bleeding experiments described in the text as group II.

Note the pronounced fall in the red cell volume at 24 hours which is continued more slowly at 72 hours and replaced by a slight rise at 7 days. Note also (after 24 hrs.) the relatively slow regeneration of albumin as compared with the rapid regeneration of globulin. Note also the general similarity in the curve when plasmapheresis was performed. For discussion of the difference in the plasmapheresis curve before 24 hours see text.

Fig. 4. The effect of replacement. The two series of curves represented above were plotted from averaged values. The broken line was made from the means shown in figures 1 and 2, i.e., the 25 standard bleeding experiments with replacement of Ringer's solution, described in the text as group I; the solid line was made from data obtained in 8 bleeding experiments without replacement described in the text as group III.

Note the similarity in the two curves, indicating the essential nature of the compensatory changes after hemorrhage regardless of whether or not the loss is replaced with Ringer's solution.

however, the changes in the albumin and globulin were similar in the two groups of experiments.

The third group of experiments was designed to determine just what part the replacement of Ringer's solution played in the hematocrit and serum protein curves. As can be seen by examining figure 4, very little difference was observed. To be sure, these animals were bled 4 and 4.5 per cent of their body weights, in contrast to the 3.5 per cent of the first group. Nevertheless, the fall in the

hematocrit value as well as in the albumin and globulin concentration was quite similar. Not recorded herein are the changes in this third group of experiments between the time of bleeding and the first hour; they were gradual and progressive, unlike the changes which occurred when blood was replaced with Ringer's solution. The immediate effect of replacement with Ringer's, as already mentioned, was a precipitate fall in hematocrit and plasma proteins, returning to a level in one hour, as the injected fluid left the circulation; the details were reported in our previous communication (1).

COMMENT. Two features of the present experiments should be emphasized. First, although the hemorrhage was severe, compensation was adequate and fatalities did not occur; in contrast, many experiments by others were associated with a fatal outcome. Second, a general anesthetic was not used. As is well known, barbiturates cause a dilatation of the spleen and a fall in the red cell volume; they also interfere with hemodilution after hemorrhage (12). Moreover, with general anesthesia, a smaller bleeding results in death in a shorter period of time (6).

Inferences from the present findings depend upon the assumption that the fall in the red cell volume measures the degree of plasma volume increase, i.e., hemodilution. This assumption seems justified by many considerations, including the following observations. In actual measurements of plasma volume with the dye method (2), the hematocrit changes reflected rather well the changes in the fluid volume of the blood. Further evidence by the same authors (13) showed a similar correlation between plasma volume and hematocrit value. Using red cells tagged with radioactive iron, a remarkable constancy was found (14) in total blood volume in dogs because "as the red cell circulating volume increases, there is a corresponding drop in the plasma volume."

Using the fall in the hematocrit value as an indication of plasma volume increase (hemodilution), it seems clear that fluid enters the blood stream after a severe non-fatal hemorrhage for as long as 72 hours, the rate being very pronounced in the first hour, less in the next 24 hours, and very slight thereafter. This phenomenon has been observed in the human (15, 2), the same period of maximum dilution (70 hrs.) occurring (2) as in the present experiments. From the fact that rapid dilution immediately followed plasmapheresis, it may be inferred that the hemodilution is not merely a phenomenon designed to replace the volume of the removed red cells; according to Starling's hypothesis, hemodilution actually may be due to a persistent fall in capillary pressure, induced by the hemorrhage.

The character of the fluid entering the blood immediately after the hemorrhage is probably protein-free, inasmuch as the concentration of protein at one hour is almost the same as the fall in red cell volume (fig. 1). The changes at 7 and 24 hours, however, indicate the entrance of fluid containing protein, whose fractions, albumin and globulin, each behave differently, particularly after the first hour. Between the first and seventh hours, it seems clear that the fluid entering the blood stream contains more albumin than globulin, inasmuch as the

concentration of albumin increases, whereas that of globulin falls (fig. 2). However, between the 7th and 24th hours, both increase. After the 24 hour period, the globulin increases much more rapidly, the albumin lagging far behind and never reaching its normal level, even after 7 days, while the globulin fraction exceeds its normal level at this period by 15 per cent (fig. 3). This difference in the behavior of albumin and globulin may be explained by assuming that the early addition of albumin to the diluting fluid is probably mobilized directly from the liver, whereas the subsequent but more pronounced addition of globulin would seem to be an actual regeneration.

On the basis of the present findings and of those of others, a biochemical approach to the problem of shock following hemorrhage seems justified. Using the term compensation to imply recovery from the effects of a severe hemorrhage, one might say that a hemorrhage is compensated when hemodilution is sufficient to restore and maintain blood volume adequately to support the circulation. Hemodilution is insufficient in maintaining blood volume only because of the low protein content of the diluting fluid.

The protein content of the fluid which restores blood volume is of decisive importance because it contributes the colloidal osmotic pressure of the blood upon which circulation and fluid interchange depend. The fact that the diluting fluid becomes poor in albumin is significant because albumin is responsible for 85 per cent of the blood's colloidal osmotic pressure.

The defect in the compensatory mechanisms would seem, therefore, to lie in the inability of the body to correct the hypoalbuminemia induced by the hemorrhage. Expressed in other words, fatalities following severe loss of blood are due to the inability of the body to restore blood volume with fluid containing sufficient albumin. This biochemical approach to the problem of shock in hemorrhage places the emphasis on an acute protein deficiency, and suggests the need for means to increase the output of albumin by the liver in order to obviate the necessity of supplying exogenous protein. Experiments along this line are now in progress.

SUMMARY

1. Hemodilution as shown by a falling red cell volume was a uniform finding in 45 non-fatal severe hemorrhages carried out without anesthesia with or without r. placement of Ringer's solution or red cells. The most pronounced hemodilution occurred in the first hour, but continued at a decreased rate for 72 hours.

2. The fall in plasma proteins accompanying spontaneous hemodilution is greatest 1 hour after the bleeding. Correction of the low albumin fraction begins rapidly in the first 6 hours thereafter, but then slows, being still incomplete at 7 days. In contrast the globulin fraction continues to fall at 7 hours, but rapidly increases to its initial value between 24 and 72 hours.

3. The concept of acute protein deficiency is offered as a biochemical explanation of the problem of fatal (uncompensated) hemorrhage; i.e., the inability of the body to supply sufficient albumin during hemodilution.

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THE RELATIONSHIP OF THE DIABETOGENIC EFFECT OF DIETHYLSTILBESTROL TO THE ADRENAL CORTEX IN THE RAT

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Diethylstilbestrol has been shown (1) to be diabetogenic in partially depancreatized and in normal force-fed rats. Since certain of the adrenal cortical hormones are also diabetogenic (2) and since the adrenal cortices hypertrophy during the administration of diethylstilbestrol (3) it is reasonable to consider the hypothesis that the diabetogenic effect of this substance is due to its stimulation of the adrenal cortex to secrete sufficient amounts of its hormones to cause glycosuria. In this study it is found that diethylstilbestrol exerts some diabetogenic effect which is not mediated through the adrenal cortex although the presence of the adrenal cortical hormones is required for the full manifestation of severe glycosuria.

METHODS. Male rats of the Sprague-Dawley strain were used. Partial pancreatectomy was carried out at a body-weight of 250 to 300 grams. All of the pancreas was removed except that portion between the bile duct and the duodenum. The technique used in earlier studies (4) was modified according to Richter (5) so that the pancreas lying within the duodenal loop was removed by suction through a small pipette. By this modification it is possible to remove up to 95 per cent of the pancreas in a single stage operation in rats of any body-weight with less than 20 per cent mortality. The technique used for adrenalectomy has been described (4).

Following partial pancreatectomy the rats were maintained on a diet of Purina dog chow until they had reached a weight of 325 to 375 grams. They were then placed in metabolism cages and maintained on a fluid diet administered by stomach tube each morning and late afternoon. The technique of force-feeding and the diets used were modifications of the methods described by Reinecke, Ball and Samuels (6). The diets used were made up according to the table (see p. 578).

During the period of adaptation to force-feeding the diets were first administered in small amounts to prevent the development of "food shock" (1). The animals were brought to a full feeding on the 8th day. Each rat received 26 cc. of diet per day. The total available carbohydrate of diet A was approximately 6.5 grams; and the total available carbohydrate of diet B was approximately 5.0 grams. These values are based on analyses of the diet and assume that the maximum formation of glucose from protein does not exceed a glucose:nitrogen ratio of 3.6.

The 11-desoxycorticosterone acetate was dissolved in sesame oil (5.0 mgm.

CONSTITUENT	DIET A	DIET B
Egg albumin (Merck)	200 grams	
Cellu flour (Chicago Dietetic Supply)	120 grams	120 grams
Osborne & Mendel salt mixture	40 grams	40 grams
Vitamin preparation (Vi-Penta. Roche)	10 cc.	
Wheat germ oil	10 cc.	10 cc.
Mazola oil	10 cc.	175 cc.
Cod liver oil	10 cc.	10 cc.
Starch	200 grams	
Dextrin	100 grams	
Sucrose	100 grams	
Butter fat	295 grams	
Dried yeast (Fleischmann)		100 grams
Whole milk powder (Merrell-Soule)		600 grams
Water to make total volume of	2000 cc.	2000 cc.

per cc.) and administered in divided doses (2.0 mgm. per day) each morning and late afternoon. The diethylstilbestrol was dissolved in sesame oil (1.0 mgm. per cc.) and doses of 0.1 mgm. were administered once daily. The adrenal cortical extract was made up in 0.9 per cent sodium chloride solution and represented 40 grams of beef adrenal glands per cubic centimeter. It was administered in divided doses (3 cc. per day) each morning and late afternoon. All solutions were administered subcutaneously. Twenty-four hour specimens of urine were collected at the same hour each day and preserved with thymol. The determination of urinary glucose was carried out according to the method of Benedict (7) and that of blood glucose by the method of Miller and Van Slyke (8).

Experiments and results. Experiment 1 was a study of the effect of diethylstilbestrol in the adrenalectomized, depancreatized rat maintained on 11-desoxycorticosterone acetate. Six rats were partially depancreatized. All of the pancreas was removed except for approximately 50 per cent of the tissue which lies between the bile duct and the duodenum. During the experiment they were maintained on diet A. Prior to adrenalectomy each of the rats spontaneously excreted glucose in amounts up to 1.8 grams daily. Following removal of the adrenal glands each animal was maintained on 2.0 mgm. daily of 11-desoxycorticosterone acetate during the remainder of the experiment and under these conditions each animal was free from glycosuria until diethylstilbestrol was administered. Following a control period of 10 days each animal was treated with 0.1 mgm. diethylstilbestrol daily until it died within a period of 5 to 11 days. During the period of treatment with diethylstilbestrol each animal developed a mild glycosuria and then, within one or two days, developed symptoms of diabetic shock and died. Determinations of blood sugar showed that symptoms of shock occurred at a time when the level of blood glucose was above 200 mgm. per cent. The data on urinary glucose are summarized in figure 1.

Experiment 2 was a comparison of the diabetogenic effect of diethylstilbestrol

in the partially depancreatized rat prior to adrenalectomy and following adrenalectomy when different types of replacement therapy were used to control adrenal cortical insufficiency. Five rats were partially depancreatized by removing all of the pancreas except the portion between the bile duct and the duodenum which was left intact. During the experiment they were maintained on diet B. Each rat was free of spontaneous glycosuria and all of them responded to the administration of diethylstilbestrol by developing a severe glycosuria prior to adrenalectomy and also during two test periods following adrenalectomy when the animals were treated with adrenal cortical extract. However, when the treatment with adrenal cortical extract was replaced by the administration of 11-desoxycorticosterone acetate only one of the five rats developed glycosuria during the administration of diethylstilbestrol. Similarily,

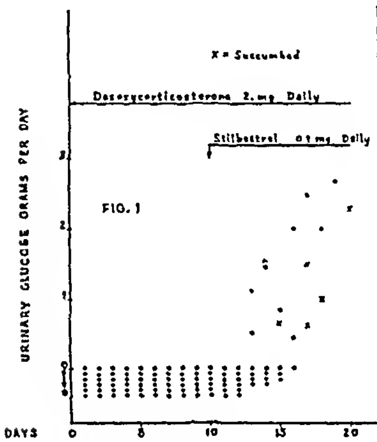

Fig. 1

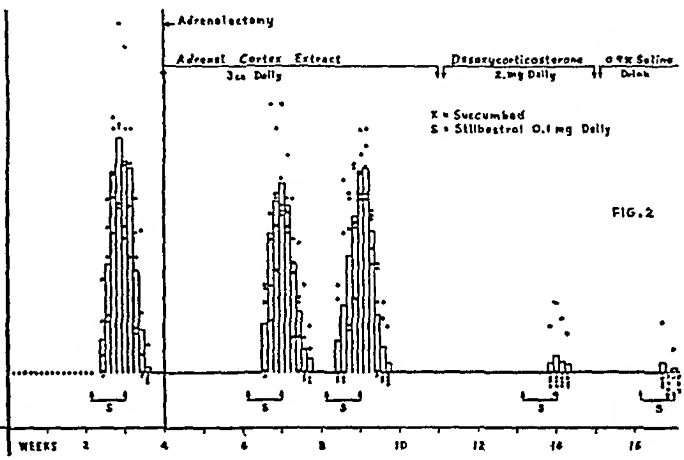

Fig. 2

Fig. 1. A diabetogenic effect of diethylstilbestrol in the adrenalectomized, partially depancreatized rat treated with 11-desoxycorticosterone acetate.

Fig. 2. The diabetogenic effect of diethylstilbestrol before and after adrenalectomy of the partially depancreatized rat as influenced by the nature of the replacement therapy.

when the animals were mantained by drinking 0.9 per cent solution of sodium chloride, two rats excreted a small amount of glucose for only one day. All of the rats died as a result of the administration of diethylstilbestrol during the period of maintenance on solution of sodium chloride. Prior to this phase of the experiment all of the rats gave the appearance of being in good health and vigor at all times. The data on urinary glucose and the periods of treatment are summarized in figure 2.

Discussion. It is clear from these experiments that diethylstilbestrol exerts some effect upon carbohydrate metabolism of the rat which is not mediated by the adrenal cortex. The presence of the adrenal cortical hormones such as occur in adrenal cortical extract is necessary for a full manifestation of the diabetogenic activity of diethylstilbestrol since glycosuria is either reduced or absent when these same test animals are maintained on 11-desoxycorticosterone acetate or by drinking 0.9 per cent solution of sodium chloride.

The amounts of adrenal cortex extract which were administered in these experiments were large as judged by the small amount of this extract which is required to maintain life in adrenalectomized rats (3 rat units per cc.) but it is well established that large doses are required to maintain normal carbohydrate metabolism in adrenalectomized rats and massive doses are required before diabetogenic effects of adrenal cortical extracts can be demonstrated (9). These experiments do not prove that none of the effect of diethylstilbestrol on the carbohydrate metabolism of the non-adrenalectomized rat is due to an increased activity of the adrenal cortex. The intensity of the glycosuria induced by diethylstilbestrol following adrenalectomy and during the period of adrenal cortex administration was less than that manifested prior to adrenalectomy. This difference may have been due to chance; it may have been due to a failure of the replacement therapy to provide as much cortical hormone as is normally secreted by the cortex; or it is conceivable that a part, but not all, of the diabetogenic effect of diethylstilbestrol is brought about by an increased activity of the adrenal cortex.

The adrenal cortex undergoes hypertrophy in animals subjected to any type of stress or damaging agent. Although it is probable that its secretory activity is increased during these periods of enlargement, proof is lacking. Similarily, it has not been proven that the enlarged adrenal cortex causes any abnormal physiologic changes in the organism similar to those induced by over-dosage of the adrenal extracts or cortical steroids. Ingle (1) studied the effect of subjecting diabetic rats to stress and damaging agents of the sort which produced marked adrenal cortical hypertrophy on the assumption that an increased secretion of adrenal cortical hormones might intensify the diabetic state just as will the injection of these hormones. The results were negative. It is conceivable that the increased secretion of hormones by the hypertrophied cortex would not intensify the diabetic state or produce other abnormal changes because under conditions of stress the increased secretion of hormones would only suffice to meet a physiologic need. Thus homeostasis would be maintained rather than being disturbed. If this hypothesis is true, the adrenal cortical hypertrophy which follows the administration of diethylstilbestrol may be necessary for a full manifestation of the diabetogenic effect of diethylstilbestrol, not because adrenal cortical hormones are being secreted in diabetogenic amounts, but because increased amounts of them are required to maintain the functional normality of those mechanisms essential for the metabolism of carbohydrate.

The failure of diethylstilbestrol to manifest its full diabetogenic effect in the absence of the adrenal cortical hormones may be due in part to the lowering of the carbohydrate stores of the body and to the high rate of glucose oxidation during adrenal insufficiency (10). Larger amounts of glucose would be required to exceed the kidney threshold for glucose in the case of the carbohydrate depleted adrenalectomized rat than in the case of the non-adrenalectomized rat. The relative importance of this and other undefined factors in causing the diminished response to diethylstilbestrol in the absence of the cortical hormones

cannot now be evaluated. It should be pointed out that the effect of pancreatectomy and of anterior pituitary extracts on carbohydrate metabolism is diminished in adrenalectomized animals but in each case can be restored by treatment with adrenal cortical extract (2).

It is significant that in experiment 1, the adrenalectomized, partially depancreatized rats treated with 11-desoxycorticosterone acetate developed shock at a time when hyperglycemia was present. Partially depancreatized rats having their adrenals intact and partially depancreatized, adrenalectomized rats treated with large amounts of adrenal cortical extract are able to resist a severe diabetic state without developing symptoms of shock. The lower than normal resistance of the animals studied in experiment 1 was therefore due to lack of some cortical hormone. Under the usual experimental conditions shock states in animals having cortical insufficiency are characterized by hypoglycemia and it is frequently assumed that depletion of the carbohydrate stores of the body is a factor in causing the low resistance of adrenalectomized animals to shock. In these experiments the animals were treated with desoxycorticosterone acetate which is believed to prevent deficiencies in electrolyte metabolism and the additional treatment with diethylstilbestrol raised the blood glucose to abnormally high levels without preventing shock. This may be taken as evidence that the effect of the adrenal cortex on resistance can be partially dissociated from deficiencies in carbohydrate and electrolyte metabolism.

SUMMARY

Six partially depancreatized rats which had a mild spontaneous glycosuria were adrenalectomized and treated with 2.0 mgm. of 11-desoxycorticosterone acetate daily. Following adrenalectomy the glycosuria disappeared but was reinduced by the administration of 0.1 mgm. of diethylstilbestrol daily. After a few days each rat died although the glycosuria was not severe. Symptoms attributable to adrenal cortical insufficiency were found to coexist with a state of hyperglycemia.

In a second experiment, five partially depancreatized rats which were without spontaneous glycosuria became diabetic during the administration of 0.1 mgm. of diethylstilbestrol daily and the glycosuria disappeared when the diethylstilbestrol was withdrawn. These animals were adrenalectomized and maintained on 3.0 cc. daily of adrenal cortical extract. Glycosuria developed only when diethylstilbestrol was administered. When these animals were maintained by treatment with 11-desoxycorticosterone acetate or by drinking a solution of 0.9 per cent sodium chloride the diabetogenic effect of diethylstilbestrol was either slight or absent.

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THE INFLUENCE OF INTERELECTRODAL DISTANCE IN ELECTRICAL STIMULATION OF NERVE AND OF STRIATED AND VENTRICULAR MUSCLE

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This study was prompted by the observation that the distance between two stimulating electrodes applied to the turtle's ventricle did not modify the threshold. As is well known, the threshold of motor nerve fibers increases markedly when the interelectrodal distance is shorter than about 5 to 3 mm. It was considered interesting to compare in this respect A and C nerve fibers, and also striated and ventricular muscle.

Method. The nerves studied were the A and C fibers in the saphenous of cats under dial anesthesia (Ciba, 0.7 cc. per kgm., intraperitoneally). The nerves were dissected, as free from fascia as possible, from the origin at the femoral nerve to below the knee; a few branches were cut in the process. The excised nerves were laid on parallel fine wires (diameter 0.2 mm.) placed at various fixed distances in a moist chamber. The stimuli were usually applied to the central part of the nerve, while the electric responses were recorded from the peripheral end; the reverse procedure, occasionally tested, yielded similar results.

With the cathode in a given position the interelectrodal distance was first increased and then decreased by using different anodes. Since the electrodes in the chamber provided only fixed distances, in order to test as many intervals as possible it was necessary to shift the cathode to different points. There were usually no significant discrepancies with the several cathodes.

The influence on the thresholds of the changes of resistance caused by varying interelectrodal distance was minimized whenever possible by putting a resistance of 10,000 to 250,000 ω in series with the nerve.

The fine wires used as electrodes were quite polarizable. A cumulative effect was prevented by placing a resistance (5,000 or 20,000 ω) in parallel with the nerve and the series resistance. Polarization led to some scatter of the measurements but not so great as to mask the phenomenon studied.

The stimuli were condenser discharges of various capacities (0.001 to 1 μF). The discharging circuit was such that the time constant of the discharges was not significantly affected by the change of position of the electrodes on the nerve. Qualitatively similar results were obtained with all the capacities tested.

The electric responses were led monophasically to a cathode-ray oscillograph after suitable amplification. They were measured visually on the oscillograph. By threshold is meant the voltage necessary to elicit a constant submaximal response (about 30 per cent of maximal).

The striated muscles studied were the sartorii of the cat and frog. They were exposed by opening the skin of the pithed frogs or the cats. Desiccation was prevented by covering with mineral oil or, preferably, by intermittent application of the appropriate Ringer's solution which was removed before the observations.

The stimulating electrodes were steel needles with a tip of about 0.1 mm. One, the cathode, was in a fixed position. The other, the anode, was supported by a mechanical stage of a microscope and could be placed with ease at any desired position. Both electrodes were applied to the surface of the muscles. A dissecting microscope with a micrometer permitted the measurement of the interelectrodal distance with an accuracy better than 0.1 mm.

The electric responses of the muscles were led to the cathode-ray oscillograph. As shown by Rosenblueth (1940), the latency of the electrograms permits the separation of responses to direct stimulation from those due to indirect activation. The direct responses were measured as above.

The ventricles, separated from the atria in pithed turtles, were pinned to a board and kept moist. The placement of the stimulating electrodes was as for striated muscle. The response observed was the ventricular contraction.

Results. The observations on A fibers confirm previous reports (see for references Rushton, 1927, 1934). The upper curve in figure 1 illustrates a typical experiment. The results are plotted in logarithmic scales. For the abscissae, this type of scale avoids the crowding of small interelectrodal distances; and for the ordinates, it allows a ready estimation of the ratio of the *threshold at a given short distance to that at long distances. The thresholds* are in conventional units.

When the changes of nerve resistance with variable interelectrodal distance could influence the distribution of current—i.e., when no resistance was placed in series with the nerve—there was a distance at which the threshold was minimal, and shorter or longer distances required higher voltage for stimulation. This was true in the experiment illustrated in figure 1; with longer interelectrodal distances than those included in the graph the threshold rose. The absence of this minimum in the observations with a high series resistance confirms Rushton's (1927, 1934) results.

The observations on C fibers in the cat's saphenous nerve were qualitatively similar to those on A fibers. Significant quantitative differences were that the threshold rise occurred only at shorter distances and that it was not as striking for the C as for the A fibers (cf. the upper and the middle curves in fig. 1). For reasons which remain obscure, the results on C fibers were more irregular than those on A fibers. The A curve was fairly constant from nerve to nerve with the same experimental conditions, while the C curve could vary markedly. Invariably, however, the effect of short interelectrodal distance was more striking for the A than for the C elements of the same nerve.

In contrast with the results obtained in nerve fibers, the interelectrodal distance had no influence on the threshold of striated and ventricular muscle (lower curve of fig. 1). Even with shorter intervals than those represented, e.g., 0.15 mm., the threshold was not different from that with a distance of 1 cm.

The problem of the influence on threshold of the angle made by the electrodes and the excitable elements is closely related to the topic of this study (see Rushton, 1927). In the sartorius muscles several observations were made in which the interelectrodal distance was constant (about 1 mm.) while the angle was varied from 0 to 90° by changing the position of the anode. No significant influence of this angle on threshold was detected.

Discussion. According to Rushton (1934) the influence of interelectrodal distance on the threshold of A fibers is due to the structure of nerves. Nerve fibers are assumed to consist of conducting cores and resistant sheaths. With

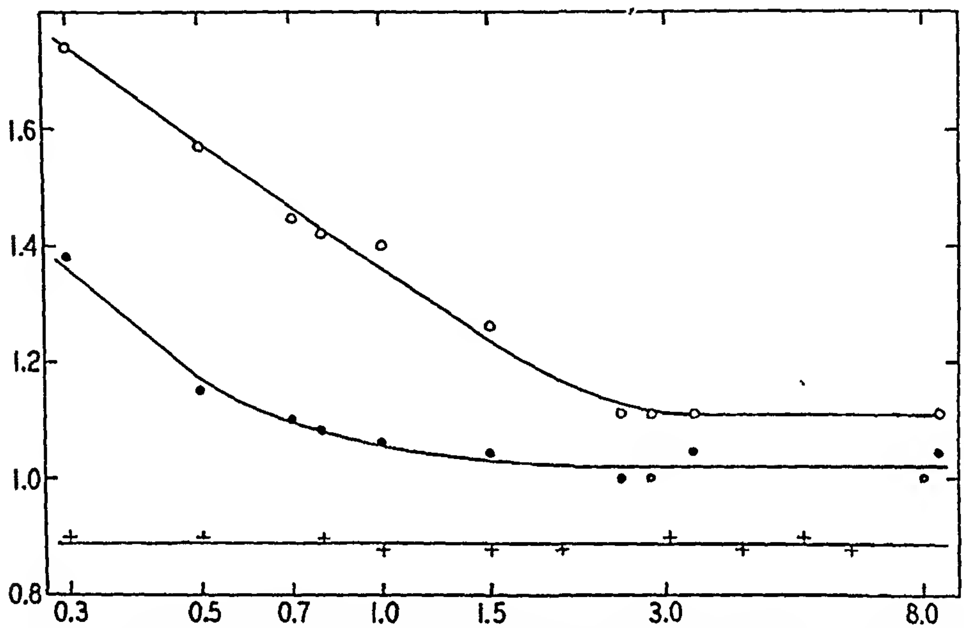

Fig. 1. Relations of threshold to distance between stimulating electrodes. Abscissae: distance in millimeters. Ordinates: threshold voltage in conventional units. The scales are logarithmic.

Upper curve (circles): nerve A fibers. Capacity of the stimulating condenser: 0.5 μF. Resistance in parallel with the nerve: 5,000 ω.

Middle curve (dots): nerve C fibers. Stimulating circuit as for the A fibers.

Lower curve (crosses): ventricular muscle. Capacity: 0.1 μF. Resistance in series with muscle: 20,000 ω. Resistance in parallel: 20,000 ω.

a long interelectrodal distance the current through the cores would be a relatively large fraction of the total current flow. With short distances, on the other hand, only a small fraction of the total current would flow through the fibers, most of it passing through the surrounding connective tissue and interstitial fluid.

The application of this theory to the present data implies that A fibers are relatively well insulated, possibly by the myelin sheath. C fibers have less insulation. Finally, striated and ventricular muscles behave as if they were practically uninsulated.

SUMMARY

With short distances between the stimulating electrodes, the threshold of C nerve fibers rises significantly, but not as much as does that of A fibers (fig. 1).

The threshold of striated and ventricular muscle to electrical stimuli is independent of the interelectrodal distance (fig. 1). The threshold of striated muscle is also independent of the angle between the stimulating current and the muscle fibers.

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EXPERIMENTALLY INDUCED HYPERTENSION IN PARABIOTIC RATS[1]

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Despite the mass of experimental data which has accumulated in recent years on the subject of hypertension, conclusions regarding the exact rôle of the kidney in this process are still a matter of dispute. The available facts have been interpreted in several ways. Some assume the liberation of a pressor substance (angiotonin, hypertensin) in the renal vein in sufficient concentration to be detectable by perfusion through an isolated rabbit's ear, or toad, or by injection into animals. Others, although accepting the existence of a renal pressor substance, do not consider that renin or its derivatives (angiotonin, hypertensin) are the agents responsible for hypertension. According to many, the relative balance between "anti-pressor" and "pressor" substances, both of which are elaborated by the kidney, determines the existence of hypertension. And finally, the hypothesis has been advanced that hypertension results simply from a deficiency of a renal factor without the implication of any pressor substance.

It is now generally admitted, however, that experimental renal hypertension is mediated by a humoral mechanism. If this be the case, the reaction of parabiotically united animals should throw light on the mechanism involved in hypertension, as it has in the case of the various hormonal reactions in which this procedure has been utilized. The blood pressures of such parabiotic rats have been determined following various operative procedures on the kidneys and the results interpreted in the light of current theories regarding the etiology of experimental hypertension.

METHODS. A total of over 50 pairs of rats have been joined in parabiosis by the technique described by Bunster and Meyer. In this procedure skin and muscles throughout the length of the animals are united, but the abdominal cavities remain separated. Animals 20 to 30 days of age, weighing 15 to 40 grams, were used. They were of a pure Wistar strain and were litter mates matched for size and sex. Successfully united pairs were allowed to attain maturity and their blood pressures determined daily throughout subsequent procedures. The blood pressure was determined on the unanesthetized animals by the plethysmographic method of Williams, Grollman and Harrison. A special holder was devised which permitted measurements to be made on each rat of a pair alternately until constant results were obtained, as in determinations on single animals.

Hypertension was induced by applying cotton cloth to the kidney. In some

[1] Aided by a grant from the John and Mary R. Markle Foundation.

cases this kidney was subsequently removed; in others the remaining normal kidney of the same rat or one of the kidneys of its co-twin were removed. In all instances, however, at least a month elapsed, during which pressures were taken daily, before proceeding to the next operation.

RESULTS. *The blood pressure in parabiotic rats.* The systolic blood pressure in the individual members of a parabiotic pair were not always identical. However, the pressure in each animal remained within a normal range and the variations between members of a pair did not exceed the daily variations observed in each individual rat. A typical series of readings taken during the course of a week on 2 pairs of parabionts is reproduced in table 1. In view of the magnitude of the variations in each individual rat from day to day, it is impossible to conclude that the variations between the members of a pair are real and not due to uncontrollable errors in the readings. However, since, as shall be shown later, the blood pressures of members of a pair may vary widely, it is probable that the

TABLE 1

The individual daily blood pressure readings on two pairs of parabiotic rats during the course of a week

Values are expressed as millimeters of mercury

Pair I:							
Right member	120	120	130	140	130	120	120
Left member	110	120	130	130	140	120	130
Pair II:							
Right member	125	130	120	125	120	120	120
Left member	110	120	130	130	120	110	120

observed differences are real and illustrate the independence of the factors regulating the blood pressure level in each rat.

The application of cloth to one kidney. As is the case in separate animals (Grollman and Williams) the application of cloth to a single kidney of one of a pair of parabiotic rats was variable in its effects. In many pairs, such an operation induced no demonstrable changes in the blood pressure of either member of the pair. In the majority of experiments, the rise in blood pressure of the rat in which cloth had been applied to one kidney was only 10 to 20 mm. of mercury, while that of the intact co-twin was within the limits of daily normal variation. In these animals the blood pressure gradually attained a maximum level 10 to 20 mm. above its preoperative level about 10 days following the operation, gradually resuming its normal level after the elapse of another 10-day period.

The third type of reaction following the application of cloth to one kidney of a single member of a parabiotic pair is reproduced in figure 1. In this group of animals the blood pressure of the rat with cloth on one kidney rose gradually to hypertensive levels and remained elevated throughout the subsequent period of observation. The blood pressure of the co-twin, on the other hand, either

remained at its preoperative level as in figure 1, or was only slightly elevated. The response shown in figure 1 illustrates this marked dissociation which may occur between the blood pressure levels of parabiotically united animals.

The application of cloth to one kidney with ablation of the other. In parabionts remaining normotensive after the application of cloth to one kidney, the removal of the remaining normal kidney from the same rat invariably resulted in an elevation of blood pressure in the operated animal to hypertensive levels (150 to 180 mm.). The rise in pressure occurred gradually during the period of a week or ten days following the nephrectomy and rose thereafter at a slow rate until the death of the animal (fig. 2). This is essentially the reaction noted in single rats following similar operations (Grollman and Williams).

The systolic blood pressure of the intact co-twin following the above-described operations was variable. In many instances it remained within normal limits

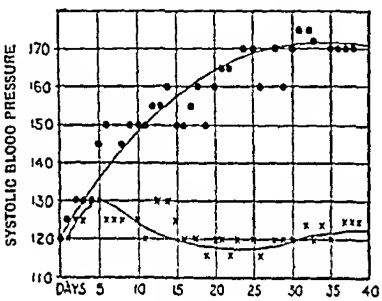

Fig. 1

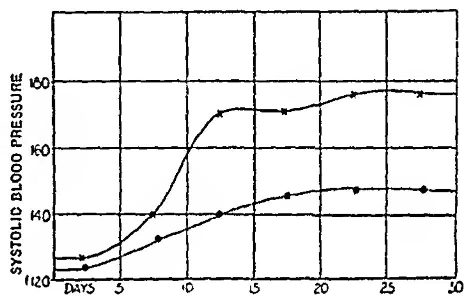

Fig. 2

Fig. 1. The effect of applying a cloth capsule to a single kidney of one of a pair of parabiotically united rats. The upper curve (—●—) shows the blood pressure response of the rat which was operated upon; the lower curve (—x—), of the co-twin, the kidneys of which remained intact. Systolic blood pressures are expressed in millimeters of mercury.

Fig. 2. The effect of applying a cloth capsule to one kidney and removing the other from one of a pair of parabiotically united rats. The upper curve (—x—) shows the blood pressure response of the rat which was operated upon; the lower curve (—●—) of the co-twin, the kidneys of which remained intact. Each point is the average of daily determinations over a period of five days. Systolic blood pressures are expressed in millimeters of mercury.

of variation, so that the blood pressure curve of the two animals was identical to that of figure 1. In most cases, however, the blood pressure of the intact animal rose moderately as illustrated in figure 2. There remained a complete dissociation of the blood pressure levels in the two animals, as in the case illustrated in figure 1.

In only one instance did the systolic blood pressures of both rats approximate the same level. In this case the average systolic pressure of the operated member of the pair was 150 mm., while that of the unoperated one rose to 140 mm.

Bilateral nephrectomy. Extirpation, which affords so valuable a method for demonstrating the hormonal function of the glands of internal secretion, cannot be applied to the kidney because of the short survival period of animals following nephrectomy. Both kidneys of one of a pair of parabiotic rats may, however, be removed and the animals remain in relatively good condition for long periods

(Hermannsdorfer). Jeffers and his collaborators have recently utilized this method and demonstrated that following the removal of three of the available 4 kidneys, hypertension results in the nephrectomized rat. We have confirmed this finding on two pairs of parabionts. In our experiments, however, the removal of the kidneys from only one rat sufficed to induce hypertension, the blood pressure of the nephrectomized animal rising to 170 to 190 mm., while that of the co-twin rose only to 130 to 140 mm. The reaction was thus similar to that shown in figure 2.

Discussion. The results of the present study demonstrate that by the procedure of parabiosis used, it is possible to maintain animals in union and nevertheless have a striking dissociation of the blood pressure of the individuals. Parabiotic union as performed results in a fine network of anastomosing capillaries penetrating between the parabionts. Although no large vessels pass between them, the exchange suffices to permit relatively rapid interchange of constituents between the blood of the two partners. This can be demonstrated by the injection of a dye into one animal and noting its rate of accumulation in the co-twin. Thus, following the injection of ½ cc. of a ½ per cent solution of Evans Blue into the tail vein of one rat, the relative amounts of dye in the blood were 54 and 46 per cent in the injected and uninjected twins, respectively, after two hours. Our results confirm those reported by Hill, using brilliant vital red, and need, therefore, not be repeated here. In the case of a rapidly excreted dye (phenolsulfonephthalein), a total of 80 per cent of the dye was excreted in three hours, 70 per cent by the injected rat and 10 per cent by its co-twin. In the case of a more rapidly destroyed substance (epinephrine) it was found that whereas this substance induced the usual pressor effect in the injected rat, no rise in blood pressure could be detected in the co-twin.

The variability in the distribution of a very rapidly utilized (epinephrine), a relatively rapidly excreted (phenolsulphonphthalein) and a slowly excreted substance (Evans Blue) between the members of a parabiotic pair exemplifies the type of reactions which one encounters in the distribution of normally excreted endocrine substances. In the case of the female sex hormone, for example, the secretion of one animal exerts only a slight effect on the estrous cycle of its partner; a normal female only rarely induces estrus in its ovariectomized co-twin; and union of a male and a female does not inhibit estrus in the latter (Hill). This demonstrates that the threshold of hormone necessary for maintaining the estrous cycle is seldom reached in the blood of the castrated parabiont. On the other hand, the hormones of pregnancy affect the non-pregnant co-twin promptly (Hill). A similar difference in the ease with which homogeneity of hormonal environment is attained in parabionts has been demonstrated by Witschi for the melanophore and metamorphosing hormones. The former is much slower in attaining its effective concentration in hypophysectomized parabiont than is the latter.

The physiological effectiveness of a transmitted humoral agent between two animals in parabiosis depends apparently on the abundance with which it is produced and its threshold concentration in the blood necessary for inducing its physiological activity (Witschi, Hill). A humoral agent produced constantly

and present in the blood in appreciable concentration, compared to its concentration in the tissues, will ultimately distribute itself between and affect both parabionts. On the other hand, if released only in small amounts and removed rapidly by the tissues, only a minimal concentration will remain in the blood and the co-twin will manifest a deficiency of the hormone in question. Assuming that the kidney elaborates a humoral substance involved in the causation of hypertension, the present experiments indicate that the kidney normally does not release an overdose of this substance but merely produces an adequate amount for the needs of the organism. The kidney apparently cannot greatly increase its output of the hypothetical substance with an increase in demand incurred by the experimental procedures employed in inducing hypertension.

At first sight, the results of the present study would seem to exclude the possibility of explaining the occurrence of hypertension by any humoral agency. However, the results obtained in the case of generally accepted endocrine reactions (*e.g.*, the phenomenon of estrus, cited above) show that it is possible to have a dissociation of effects on the members of a pair of parabionts even in the case of humoral substances, if the latter be present in the blood in amounts much less than the threshold level necessary for inducing its specific action. The present experiments are compatible with the views that the kidney elaborates 1, pressor, or 2, anti-pressor substances, or 3, a humoral agent in the absence of which hypertension results.[2] The results, however, do exclude the possibility that any humoral agent involved is present in the blood stream in sufficient concentration to induce its effects directly.

The present findings are consistent with the view that the kidney elaborates a substance which is essential for the organism and in the absence of which hypertension results, or that such a substance exerts an "anti-pressor" activity to antagonize the action of some pressor substance. The fact that bilateral nephrectomy alone of one of a pair of parabionts results in an elevation of blood pressure, would favor the first and simpler view, for it is difficult to see how simple ablation of renal tissue could induce an imbalance between the amounts of "pressor" and "anti-pressor" substances.

SUMMARY

Rats were joined in parabiotic union and their systolic blood pressures determined daily following operative procedures on the kidney. Parabiotic individuals retain an independence of their circulatory adjustments; hypertensive blood pressure levels may be maintained in one member of a parabiotic pair, while the blood pressure of the co-twin remains normal. In some instances, however, the hypertensive action induced by procedures on the kidney is trans-

[2] The view that hypertension results from the absence of some essential constituent from the organism may appear incongruous on superficial consideration. However, it is analogous to the rise in blood pressure which occurs in anoxemia, where it is the absence of oxygen which induces changes which in turn cause the observed change in blood pressure. Similarly, the lack of some incretory product of the kidney (Grollman) may result in changes in the organism which in turn give rise to hypertension.

mitted to the intact co-twin. The results are interpreted as being most consistent with the view that the kidney normally elaborates a substance necessary for the maintenance of normal blood pressure levels. The bearing of the results on other current theories of the pathogenesis of experimental renal hypertension is discussed.

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VENOUS PRESSURE AND CIRCULATION TIME DURING ACUTE PROGRESSIVE ANOXIA IN MAN[1]

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Information concerning the venous pressure and the circulation time during anoxia in man is limited and inconclusive. Schneider and Truesdell (1), using the indirect method of Hooker and Eyster (2), found a decrease in the venous pressure during the acute anoxia induced by rebreathing, or by dilution of the respired air with nitrogen, or by reduction of the atmospheric pressure in a chamber. The decrease was attributed to splanchnic vasodilatation. Levy, Barach and Bruenn (3) observed the effects of breathing a mixture of 12 per cent oxygen and 88 per cent nitrogen for a period of approximately twenty minutes. In eighteen patients with heart disease, the arm to tongue circulation time, with two exceptions, was reduced. In eleven normal subjects relatively small changes in the circulation time were noted, although ten showed a decrease. There was no definite trend of the variations in the direct venous pressure in the abnormal subjects; it was not measured in the normals.

METHODS. Nineteen normal volunteers from the enlisted personnel stationed at Randolph Field, Texas, were subjected to progressive anoxia induced by rebreathing. In the rebreather[2] the utilized oxygen was automatically replaced by nitrogen and the carbon-dioxide was absorbed by soda-lime.

The venous pressure was measured continuously by the Moritz von Tabora direct method (4) in an antecubital vein of the right arm. An 18 gauge needle was used in the first few experiments; in the remainder a 15 gauge needle was used. The skin over the selected vein was anesthetized with a 2 per cent solution of novocain before introducing the needle. The arm was placed on the bed at the same level as the subject's body. The zero level of the manometer was set 5 cm. below the fourth right costo-chondral junction. A 5 per cent solution of sodium citrate was the anticoagulant. The entire system was kept free of clots by frequent small irrigations with citrate solution stored in a reservoir connected with the apparatus.

The circulation time from the right arm to the tongue was determined by the decholin method (5). The amount of decholin used varied from 3 cc. to 5 cc. of a 20 per cent solution. It was injected rapidly and the time was measured by a stop watch from the end of the injection until the subject first perceived a bitter taste.

Arterial pressure was determined by the auscultatory method with a mercury

[1] Presented at the Thirteenth Annual Meeting of the Aero Medical Association of the United States, at Boston, Massachusetts, November 1, 1941.

[2] Designed and constructed by Lt. Col. N. W. White, M.C., U. S. Army.

manometer. Heart rates were counted with a stop watch and frequently recorded by the electrocardiograph. During the final five minutes of each rebreathing period and for the first few minutes after the subject again breathed room air, the electrocardiogram was recorded continuously.

Oxygen saturation of the ear blood was determined continuously throughout the course of each experiment by the photocell oximeter of Millikan (6). Checked by chemical analyses this method has been shown to have an accuracy within 5 per cent down to arterial oxygen saturations of 75 per cent, and within 8 per cent below that level. With this method the normal oxygen saturation is assumed to be 96 per cent.

Respiratory volumes were determined from the spirograms. These were corrected for dry air at 0 degrees C. and 760 mm. of mercury pressure.

Each experiment was conducted in an air conditioned room at a temperature of approximately 27°C. (80°F.) as follows: The subject reclined for at least thirty minutes before the rebreathing was started. During this time all variables except the pulmonary ventilation were measured. During the rebreathing period, which varied from twenty to twenty-five minutes in length, the oxygen saturation of the ear blood, the venous pressure, and the pulmonary ventilation were recorded continuously. The pulse rate and the arterial blood pressure were determined at frequent intervals. The circulation time was measured when the oxygen saturation was approximately 85 per cent and again when it was 75 per cent. At the end of the rebreathing period the subject breathed room air. All variables except the pulmonary ventilation were again measured for at least five minutes or until they had reached basal levels.

The volume of the packed red blood cells was determined in seventeen subjects at various levels of anoxia down to 75 per cent oxygen saturation of the blood. In fourteen of these individuals the changes were slight; in three they were marked. Since the data seem insufficient for any conclusion, further reference to them will be omitted at this time.

Results. Figure 1 shows one type of response to the anoxia induced by rebreathing. In subject 18 (fig. 1) there was a sudden drop in the systolic, diastolic, and pulse pressures and an abrupt rise in the venous pressure when the oxygen saturation of the ear blood was 64 per cent. At the time the subject complained of feeling faint. In this series seven subjects (37 per cent) showed a similar "circulatory crisis."

Subject 20 displayed a rise in the systolic and pulse pressures, and a gradual fall in the venous pressure (table 1) as the oxygen saturation of the ear blood approached 65 per cent. The rebreathing period was terminated because the subject showed some clouding of consciousness. This "non-fainting" type of reaction to anoxia was observed in twelve subjects (63 per cent).

Venous pressure. The results of eighteen experiments are shown in table 1. In each subject the initial or control venous pressure is the average of at least five readings over a period of more than fifteen minutes. The figures show no particular trend of the venous pressure with increasing anoxia. Figure 2 shows five of the patterns of venous pressure observed. Subject 16 showed a fairly

progressive fall. A similar pattern was seen in only four other individuals (3, 11, 13, 20) in this group (28 per cent). The remainder displayed a response similar to one of the upper four patterns in the figure. There was no predominance of any one pattern.

In the seven subjects (37 per cent) who showed a "circulatory crisis" with anoxia, a terminal, precipitous rise in venous pressure was observed simultaneous

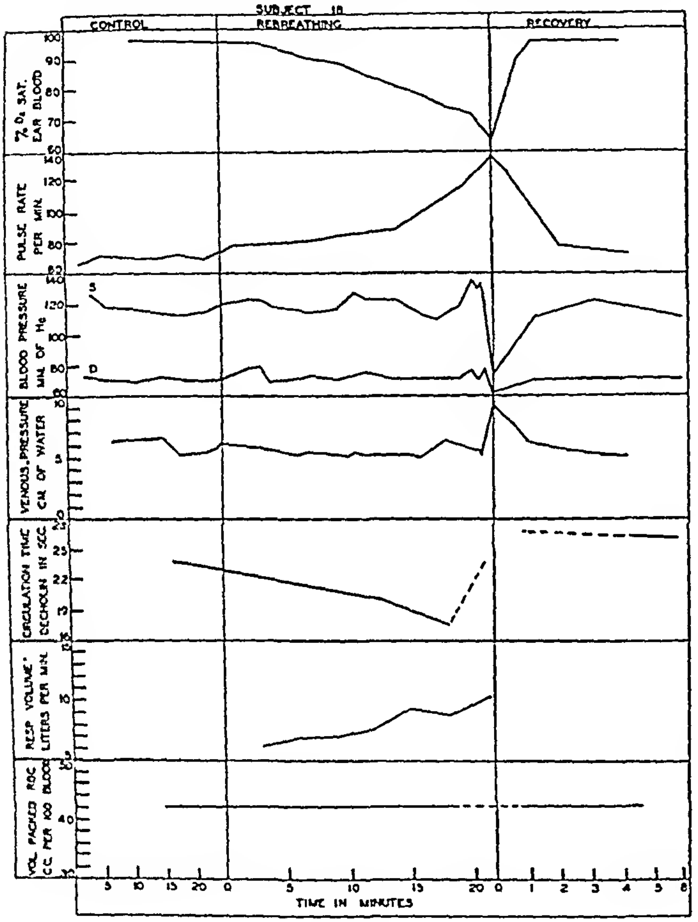

Fig. 1

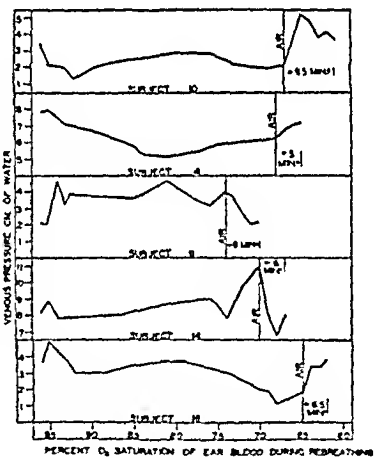

Fig. 2

Fig. 1. Circulatory measurements, respiratory volume, and volume of packed red blood cells during progressive anoxia in a normal white male, age eighteen years. The experiment was terminated because of syncope ("fainter").

Fig. 2. Direct venous pressure in five subjects during progressive anoxia. The vertical line labelled "air" at the right end of each chart indicates the point at which the subject was again permitted to breathe room air. The time during which venous pressure determinations were continued during the recovery period is also indicated on each chart.

with the onset of this crisis (table 1, subjects 2, 5, 8, 13, 14, 15, 18). A similar response has been observed in anoxic dogs (7).

The circulation time from the right antecubital space to the tongue was shortened in eighteen subjects during anoxia (table 2). In all but two (subjects 5, 13) this time was shorter when the oxygen saturation of the ear blood was 75 per cent

than when it was 85 per cent. Compared to the control, the greatest reduction when the oxygen saturation was 85 per cent was 7.2 seconds (subject 15); when the oxygen saturation was 75 per cent it was 13.0 seconds (subject 6).

In twelve subjects the circulation time could be ascertained in all four predetermined periods (table 2). In these twelve the average time during the control period was 19.2 seconds; with an oxygen saturation of 85 per cent and 75 per cent it was 16.3 seconds and 12.9 seconds respectively; and in the stage of recovery it was 21.7 seconds. The differences in these averages were found to be statistically significant except when the mean of the control period was compared to the mean of the recovery period.

TABLE 1

The venous pressure in centimeters of water in eighteen normal subjects during acute progressive anoxia

The oxygen saturation was measured in the ear blood by the photocell oximeter

O_2 SAT.	SUBJECT																		MEAN
	2	3	4	5	6	7	8	9	10	11	13	14	15	16	18	19	20	21	
%																			
Control (96)	6.1	8.1	7.7	8.5	2.1	2.6	7.6	5.1	3.5	3.3	3.5	8.1	5.5	3.5	5.9	8.4	6.6	6.9	5.7
95–92		8.0	7.5	10.0	3.5	3.1	7.9	5.7	2.2	2.5	3.6	8.3	6.2	3.8	5.6	8.3	7.3	7.2	5.9
91–87	6.8	8.2	6.4	9.3		2.9	7.2	5.4	2.4	3.3	3.3	8.0	5.8	3.0	5.3	8.5	7.3	7.2	5.9
86–82		7.6	5.6	8.8	3.5	3.2	7.0	5.6		1.6	2.7	8.6	5.6	3.5	5.2	9.6	5.9	7.2	5.7
81–77	7.6	6.6	5.4	12.2	4.1	5.0	7.2	5.1	2.8		2.7		5.3	3.6	5.1	9.1	4.1	6.7	5.8
76–72	12.5	6.8	5.8		3.2		7.8		2.5	2.6	3.0	8.5	4.8	3.0	5.9	10.4	4.2	7.6	5.9
71–67			6.2				7.4	6.0	2.3		2.8	10.4	4.8	1.2	5.3		3.4	8.1	5.3
66–62							9.4	6.4			3.4		8.4	1.8	9.4		3.8	8.1	6.3
Recovery (96)	9.5	6.8	7.0	8.2	2.3	3.9	7.5	5.5	4.3	2.7	3.3	7.3	5.2	3.6	5.5	8.6	6.1	7.0	5.8

TABLE 2

The right arm to tongue circulation time (decholin) in eighteen normal subjects before, during and after progressive anoxia

O_2 SAT.	SUBJECT																		MEAN
	2	3	4	5	6	7	8	9	10	12	13	14	15	16	18	19	20	21	
Control (96%)	25.4	22.5	19.0	24.4	26.2	13.4	10.0	22.8	23.3	24.0	14.0	14.3	29.2	17.0	23.0	24.0	19.0	14.6	20.3
85%	24.0	21.5	18.6	17.4	20.8	10.8	9.1	17.3	16.8	22.2	8.7	11.8	22.0	15.0	20.0	16.8	16.6	12.8	16.8
75%	B	15.0	B	20.2	13.2	B	8.2	14.6	16.2	B	10.2	8.6	18.6	14.5	17.5	11.2	14.0	11.7	13.8
Recovery (96%)					22.2	17.6	10.6	22.3	18.8	32.2	16.0	16.5	32.2	20.9	26.5	26.5	23.0	15.0	21.4

B = blank—the subject was unable to taste the injected drug.

DISCUSSION. The incidence of "fainting" and of "non-fainting" reactions in this small group is comparable to the results of Schneider's studies (8). He found 47 per cent in the first category, and 53 per cent in the second, compared to an incidence of 37 per cent and 63 per cent respectively in the present series. The usually accepted explanation for the circulatory crisis seen in some subjects is a failure of the peripheral vasomotor mechanism. The abrupt rise in the peripheral venous pressure that invariably accompanies such a reaction suggests rather that acute failure of the right ventricle is the principal cause (9).

The considerable shortening of the circulation time observed, in contrast to the results of Levy, Barach, and Bruenn, may be attributable to the different techniques used. The latter determined the rate of circulation before and at the end of a period of twenty minutes during which the subjects breathed a mixture of 12 per cent oxygen and 88 per cent nitrogen. It is possible that when the anoxia is not progressive, compensations may occur after twenty minutes which restore the rate of the circulation to its control value.

The average circulation time during the control period in our eighteen subjects was 20.3 seconds. In ten it was above this average, and in one its duration was 29.2 seconds. The usually accepted limits for circulation time determined by the decholin method is 10 to 16 seconds, and the average time in normal, basal subjects is 13 seconds (10).

The technique used in the present series differed from that used in others in that the needle was placed in the vein after the induction of local anesthesia. Further, the needle was left in situ and the subject was recumbent for at least 15 minutes before the first determination. The experiments were done late in the afternoon.

In order to explain these slow rates in normal subjects some further experiments are in progress, the results of which indicate that the whole matter of circulation time must be reinvestigated with a consideration of such variables as the posture of the subject, the position of the arm with respect to the thorax, the size of the needle, the effect of repeated venipuncture, the pulse rate, the blood pressure, and others. From a few preliminary experiments it appears that a simple venipuncture of itself can shorten the rate of the circulation from the arm to the tongue by as much as five seconds. If this puncture is accompanied by much trauma to the vein the circulation time may be greatly prolonged, possibly from reflex vasoconstriction as well as from venospasm caused by the direct stimulus. A precise explanation for the slow rates observed is lacking, but since the majority of these checked quite closely with the rate of the circulation determined at the end of the experiment after the subject breathed room air for a brief interval (table 2), the relative changes observed during anoxia appear valid.

In four instances (table 2, subjects 2, 4, 7, 12) the bitter taste was not perceived by the subject when the oxygen saturation of the ear blood was 75 per cent. Since anoxia is known to depress the acuity of various senses, it is probable that in these subjects the end organs of taste or the cerebral receptive centers failed to respond. If this depression occurred in the other experiments but to less degree, the figures on the circulation time are too high, and the rate is increased even more than indicated by these experiments.

CONCLUSIONS

In nineteen young, healthy male subjects, acute progressive anoxia, induced by rebreathing, had the following effects:

1. The venous pressure showed a variable response. In four subjects it progressively decreased. In seven subjects who fainted during the rebreathing,

the venous pressure rose precipitously just before syncope, suggesting failure of the right ventricle. In all cases the venous pressure was restored promptly to normal by permitting the subject to breathe room air.

2. The circulation time from the right arm to the tongue was decreased in all subjects. This decrease was statistically significant. The rate of circulation was normal or slightly slower in some cases as soon as the oxygen saturation of the blood was restored to the control level.

The authors are indebted to Riedel-de-Haen, Inc. for the supply of decholin sodium used in these studies.

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THE REPUTED RESERVOIR FUNCTION OF THE SPLEEN OF THE DOMESTIC FOWL

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It was demonstrated by Barcroft (1) that the spleen of mammals serves as a reservoir of erythrocytes and that in times of emergency it expels the reserve cells into the general circulation. This function has been ascribed, also, to the avian spleen by Harmon *et al.* (2). They determined the blood hemoglobin of seven adult fowls before and after asphyxia with the spleen intact and after splenectomy. The birds were placed in a darkened coop and allowed to remain undisturbed for one hour after which time the first blood samples were taken from the wing veins. Immediately after taking the first sample, "asphyxia was induced by pinching the trachea from the outside until the bird went limp." The second sample was then taken quickly from the opposite wing vein. Existence of the reservoir function of the spleen was considered proven if the blood sample following asphyxia (second) showed an increase in hemoglobin over the sample before asphyxia (first) when the spleen was intact, and if no increase in the second sample over the first was observed after removal of spleen. The determinations were based, apparently, upon only one sample per bird and were made by means of a Dare hemoglobinometer. The figure reported represented an average of ten readings of the same sample. Later, Harmon (3) reported the presence of significant hemoglobin reserves in the spleens of young cockerels, immature pullets and mature hens (laying hens) but not in cocks and broody (non-laying) hens.

The reliability of Harmon's data may be questioned because it has been shown that the Dare hemoglobinometer is not suitable for the determinations on chicken blood. Moreover, the structure of the spleen in birds differs from that of mammals in characteristics unfavorable to the rapid and regulated expulsion of erythrocytes. These facts indicated the need for further study on the problem.

Methods. In the following experiment, the procedure employed in taking the blood samples was not different from that of Harmon *et al.* except that instead of inducing asphyxia immediately after taking the first sample, an interval of approximately one hour elapsed before asphyxia was induced, after which time the second sample was taken. During this period, the birds were kept in a darkened coop which afforded no opportunity for disturbance. This change in procedure, it is believed, should have no adverse effect on the spleen in exerting its alleged reserve function. The samples were taken at weekly and bi-weekly intervals. All of the birds were laying before splenectomy and most of them continued to lay after splenectomy.

A number of methods have been employed in making hemoglobin determinations on fowl blood, and considerable variation in the values thus obtained resulted. The most reliable one is the Newcomer acid hematin method as modified

by Schultze and Elvehjem (5). This modification eliminates the turbidity of solution produced by the nucleated red cells of chicken blood, which is a common source of error for the unchanged method. The procedure followed in this study varied from that of Schultze and Elvehjem in that after dilution of the blood sample (0.01 cc.) with 5 cc. of 0.4 per cent NH_4OH, an equal amount of HCl, of appropriate dilution, was added. The hemoglobin of the acid solution was then determined with a photoelectric colorimeter. The results obtained are in close agreement with those of Schultze *et al.* (6).

Splenectomy was performed by making an incision on the right side of body between the last vertebral ribs. After blunt dissection and manipulation of the viscera, the spleen was exposed. A pair of cup-shaped forceps which could be extended around and under the organ was used. A loop of thread, slipped over the forceps and under the spleen was drawn together, thus ligating all blood vessels attached to it. The organ was then enucleated with fingers. Of a total of 26 birds, four died as a result of the operation. Blood samples were taken at six weeks to two months after the operation.

RESULTS. The hemoglobin determinations for the various hens before and after splenectomy are shown in table 1, and the analysis of variance of these values is presented in table 2.

Before splenectomy. The results are based upon a total of 44 hemoglobin determinations for each of the samples before and after asphyxia, or upon four paired samples per hen. The means for the samples before and after asphyxia (table 1) are 8.90 and 9.12 grams per 100 cc. of blood. That the difference in these means is not significant is revealed by the insignificant mean square 1.08, for before and after asphyxia (table 2). When the sources of variation are segregated, it is observed that a significant portion of the difference in the above means is due to the variances between samples per hens and between hens. The interaction, which is not significant, indicates that the hens responded in a similar manner before and after asphyxia.

Of the 44 determinations after asphyxia, 26 showed a slight increase, in most cases less than 10 per cent, over the first samples, 16 showed a decrease and two of them were unchanged.

After splenectomy. The difference in the means of the samples before and after asphyxia (9.03 and 8.95 grams per 100 cc., respectively) is not significant, while the variances between hens and between samples per hen are significant. The interaction was omitted here because of unequal frequencies in some of the classes.

Of the 42 determinations after asphyxia, 24 represented a decrease in amount of hemoglobin over the first sample, 14 represented an increase and four were unchanged.

Hemoglobin determinations on first and second samples with spleen intact and no asphyxia. These analyses were made in order to determine the variation in hemoglobin between first and second samples of blood per hen taken under conditions in which the splenic reserve supposedly does not function. The samples were obtained from the same eleven hens (table 1) which were used for the splenic reserve study. An interval of one hour elapsed between collection of first and

TABLE 1

Hemoglobin in grams per 100 cc. of blood

HEN NO.	WITH SPLEEN INTACT		AFTER SPLENECTOMY		HEN NO.	WITH SPLEEN INTACT		AFTER SPLENECTOMY	
	Before asphyxia	After asphyxia	Before asphyxia	After asphyxia		Before asphyxia	After asphyxia	Before asphyxia	After asphyxia
1	9.90	11.05	9.55	9.55	7	11.50	12.60	9.90	9.25
	11.50	11.80	10.50	10.35		9.80	10.00	10.60	9.80
	10.00	10.35	11.20	10.35		10.30	9.80	10.50	11.00
	9.55	9.80	10.55	10.20		8.60	8.25	10.05	9.80
2	7.65	8.70	10.00	10.70	8	9.35	10.75	10.30	9.55
	9.25	8.80	9.30	8.75		9.75	10.85	10.30	9.75
	7.60	8.05	9.50	10.05		9.10	10.35	9.10	9.00
	8.25	8.05	9.55	9.10		9.55	9.30	9.55	9.25
3	8.60	8.10	6.90	6.50	9	9.30	9.80	9.05	9.55
	6.75	7.40	7.05	7.10		9.80	9.55	8.60	9.55
	6.60	7.15	9.55	9.55		9.30	9.20	8.05	8.05
	7.20	7.25	9.70	10.05		8.65	7.85	8.60	8.05
4	8.15	8.85	8.30	8.10	10	9.75	9.65	9.75	9.75
	9.55	9.15	7.60	7.00		10.00	10.75	10.05	9.15
	8.65	10.00	5.20	5.10		9.25	9.80	10.85	10.30
	8.80	9.05				9.95	9.75	9.55	10.20
5	9.05	9.05	7.55	8.10	11	8.70	9.10	6.40	6.50
	10.05	9.05	9.15	8.75		9.95	9.55	8.65	9.05
	7.40	7.65	10.15	10.05		9.10	9.35	9.40	8.70
	7.15	7.55				9.10	8.60	8.80	9.55
6	7.15	6.65	8.85	8.60					
	8.00	8.00	8.65	7.85	Means	8.90	9.12	9.03	8.95
	7.10	7.45	7.05	7.85					
	6.95	7.60	5.55	6.50					

TABLE 2

Analysis of variance of the hemoglobin values for hens before and after asphyxia, with spleen intact and after splenectomy

SOURCE OF VARIATION	BEFORE SPLENECTOMY			AFTER SPLENECTOMY		
	D.F.	Sums of squares	Mean squares	D.F.	Sums of squares	Mean squares
Total	87	135.02		83	151.55	
1. Before and after asphyxia	1	1.08	1.08	1	0.15	0.15
2. Between samples per hen	33	92.75	2.81*	31	87.45	2.82*
3. Between hens	10	89.83	8.98†	10	86.25	8.62†
4. Interaction	10	1.84	0.18			
5. Error	33	42.27	1.28	41	64.10	1.59

* Significant on 0.05 level.

† Significant on 0.01 level.

second samples, and during this period the birds remained at rest in a darkened coop. When they were removed for taking of samples, particular care was exercised in handling to prevent undue excitement.

The results are based upon 31 determinations each for the first and second samples, or in most cases, upon 3 paired samples per hen, and these were taken at weekly intervals. The mean hemoglobin in grams per 100 cc. for the first sample was 8.80 and for the second sample 9.02. The difference in these means is nearly the same as the difference between the means before and after asphyxia with spleen intact and is not significant. The variances between the different hens and between samples per hen are significant, as was true in the previous analysis.

Discussion. These results, contrary to those of Harmon *et al.*, reveal that asphyxia does not stimulate the spleen of the fowl to expel erythrocytes into the general circulation, as it does the spleen of mammals. It is known that the high degree of contractility of the mammalian spleen plays an important rôle in the expulsion of blood into the main circulatory channels (Barcroft, 1; Klemperer, 4), and that such contractability is dependent upon the thick muscular capsule and prominent trabeculae of the organ. The spleen of birds has a thin capsule with few muscle fibers and no true trabeculae (Klemperer, 4) and is capable of contraction only to a slight extent. From anatomical considerations, therefore, it is not surprising to find the splenic reservoir function absent in the fowl.

SUMMARY

1. No evidence was found for the existence of a reservoir function of the spleen in the domestic fowl. The means for the blood samples before and after asphyxia when the spleen was intact were 8.90 and 9.12 grams of hemoglobin per 100 cc. of blood. The means for the samples after splenectomy were 9.03 and 8.95 grams respectively. The differences in these means are not significant.

2. The means for the first and second samples (no asphyxia) with the spleen intact were 8.80 and 9.02 grams of hemoglobin respectively per 100 cc. of blood. These means are nearly the same as the means for the samples before and after asphyxia before removal of spleen, and are not significant.

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REGIONAL RELATIONSHIPS OF RATE OF WATER LOSS IN NORMAL ADULTS IN A SUBTROPICAL CLIMATE[1]

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It is well known from ordinary methods of clinical examination that there are variations in the rate of sweating from skin surfaces from different portions of the body. Observers have made attempts to measure these differences quantitatively. In most instances the methods employed were inaccurate. Galeotti and Macri (1) found the rate of insensible perspiration to vary from 60.1 mgm. per 10 sq. cm. of skin per hour of the palm to 10.6 mgm. on the medial region of the abdomen with the skin areas of other regions of the body having rate varying between these extremes. Several years later Kuno and his associates (2) measured the rate of insensible perspiration from the same areas and found essentially the same values. These and other values have been discussed by Kuno (2) and need not be presented in detail again.

The studies to be reported were made in order to determine the rate for a subtropical climate and also to learn whether or not there were any differences due to season when the subjects were studied under constant laboratory conditions.

Methods and materials. The measurements were conducted in an air-conditioned room, maintained at 75°F. ± 1° and relative humidity 50 percent ± 2 percent as a comfortable environment. To make the room hot and humid in order to stimulate visible sweating the temperature and relative humidity were increased to 105°F. ± 2° and 75 percent ± 2 percent respectively. There were no perceptible currents of air at any time. The flow of air was less than 20 feet per minute. The room was so designed and decorated to reduce psychic disturbances to a minimum (3). The subject did not have any contact with the observers and the greater part of the apparatus, once the small cups (fig. 1) had been sealed to the skin to pick up the water.

The method, previously described (4), consists essentially of conducting dry oxygen from a supply tank into chambers enclosing the digits or area of skin. There the water from the surface of the skin is vaporized and conducted through aluminum coils where it is trapped by freezing. The amount of water lost during a known period of time is determined from the differences in weight of the coils before and after the water is condensed. The chambers used to enclose the finger and toe tips are more or less similar to those previously illustrated (4). The brass chamber used for collecting the water from surfaces of skin such as on the forearm or abdomen is shown in figure 1. It consists of a chamber *c*, into which the dry oxygen enters. This oxygen then enters chamber *e* through 10

[1] The studies were supported by a grant from the Rockefeller Foundation.

openings, *d* radially placed in order to ensure thorough distribution. In *e* the dry oxygen collects the water from the surface of the skin. The water laden oxygen then flows through the large orifice, *b*, and through tubing, *a*, to the collecting coil, where the water vapor is condensed and weighed. The chamber is light and insures an even distribution of the dry oxygen with adequate collection of the water from the skin. The edges of the chambers which are resting on the skin can be shaped to conform with the part to be covered. The chambers are sealed to the skin with a water soluble rubber cement (Flex-O-Fix) which dries within 15 minutes without contracting and tugging the skin. Most of the cements tried pulled the skin and interfered with the underlying circulation.

The studies were conducted as follows: The subject entered the observation room which had already had its atmosphere adjusted to a temperature of 75°F. and a relative humidity of 50 percent. The patient removed all of his clothes except his underwear, entered a comfortable bed with an inner spring mattress and covered with cotton sheets or a woolen blanket, to suit his comfort. The chambers enclosing the parts were sealed in place and after a period of approximately 45 minutes the water lost from the enclosed areas of skin was measured.

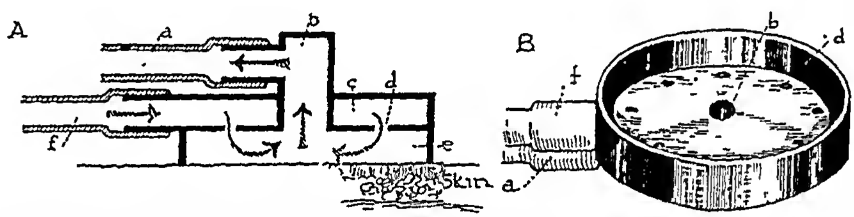

Fig. 1. Diagram of the metal chambers that were sealed to the skin to collect the water of sensible and insensible perspiration. They were built to cover variable areas of skin, usually 5 or 10 sq. cm.

The water was collected continuously throughout the entire period of study. By turning stopcocks and diverting the water laden oxygen from one collecting coil into another, the water loss could be separated into samples of 15 minute periods. Two or three collections of 15 minute periods each were made and then without the subject's knowledge the thermostat and humidistat were readjusted and the room temperature and relative humidity increased to 100°F. and 75 percent respectively. In about 15 minutes the atmospheric conditions of the room reached these new levels. During this period of change and for 30 minutes after the new level was reached the water loss from the areas of skin was measured so that at least two 15 minute collections were made with the room hot and humid.

The rate of water loss from the skin was measured during the winter and early spring months (January to March inclusive)[2] and again during the hot and humid summer months (July to August) in New Orleans. The conditions of the experimental room were the same during both seasonal periods. As many of the same subjects were used during each season as were available.

[2] Average temperature and relative humidity for New Orleans for the period of January to March, 1942, inclusive, were 55.1°F. and 68.6 per cent respectively and for July and August 1942, 83.0°F. and 80.3 per cent.

No attempt was made to make the determinations with the subjects in a post-absorptive state. They were advised to eat a light breakfast or lunch and were then studied about 2 to 3 hours later so that digestion was at a low ebb during the observations.

The studies were conducted on 46 normal adults, 14 of whom were females and 32 males. Forty-two were white and 4 negro. They varied in age from 20 to 53 years, only one being above 45 years. Thirty-seven subjects were studied during the winter season and 12 during the summer.

The 17 areas of skin studied were that over the right index finger tip, right second toe tip, right forearm (volar surface), external surface of the right forearm, epigastrium, anterior surface of the right thigh, posterior surface of the left thigh, area over the right cheek, forehead, right axilla, left axilla, posterior surface of the right hand, posterior surface of the left leg, right flank, plantar surface of the heel of the right foot, plantar surface of right foot in the region of the heads of the metatarsals, the mid-plantar surface of the right foot, and the palm of the right hand. Three or four areas were studied simultaneously. When the observations were repeated in the summer months only a few of these areas of skin were selected for study.

Results. A total of 691 separate 15 minute determinations were made on the 46 subjects.

The rate of water loss from areas of skin was expressed in milligram per 10 sq. cm. of skin area, per 15 minutes. These units will not be repeated, only the numerical values will be given.

The rate of water loss was found to vary markedly from area to area, from patient to patient and from time to time in the same patient. Figures 2 and 3 summarize the mean and extreme values for 17 different areas of the body.

The mean and extreme rates of insensible and sensible water loss respectively for the various parts of the subjects studied during winter and early spring months are shown in figure 2.

The rates of water loss determined in subjects during the summer months for the right index finger tip, right second toe tip, right forearm, middle of the forehead and mid-epigastric region are summarized in figure 3. It can be seen that the values obtained in the summer months are approximately the same as those measured during the winter months. The only part that appears to show a significant difference is the volar surface of the forearm, the rate being greater during the summer. With a study of many more subjects, this difference may be lost.

The individual variations are great and the variations in the same individual from one 15 minute period to the next may be large. Figure 4 illustrates these variations for two parts, namely, the right index finger tip and the right forearm. The variations from subject to subject are greater when he is in a hot and humid room than when the room is comfortable. Figure 5 illustrates variation from one 15 minute period to the next over a period of one hour when the subject rested in a comfortable room. There are no prolonged studies for a hot and humid room.

The rate of water loss from the right index finger tip, volar surface of the right forearm and the anterior surface of the right thigh of one subject was studied, in

which the temperature and relative humidity were raised from 75°F. and 50 percent respectively to 95° and 75 percent, to 100° and 75 percent. The atmospheric conditions were maintained sufficiently long for the water loss to reach a level before the conditions were changed. When the temperature and relative humidity were increased to 95° and 75 percent, an increase of 20° and 25 percent respectively, the rate of water loss is increased slightly and a new level is reached

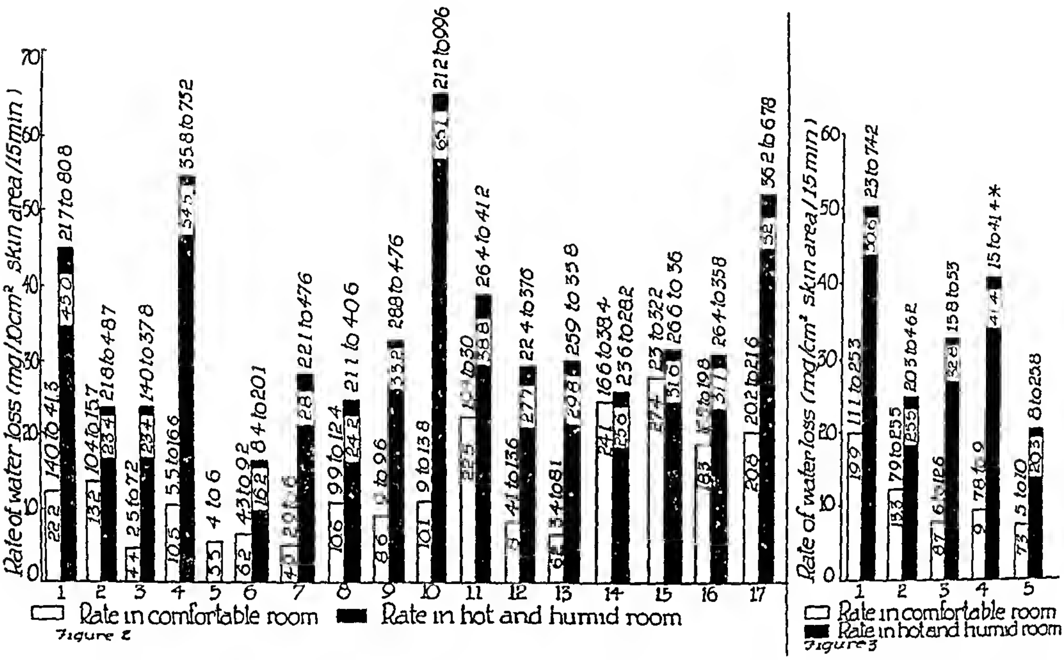

Fig. 2. The mean and extreme values for the rate of water loss from 17 different skin areas of 37 normal adults obtained during the months of January through March. The parts studied are represented as follows by the numbers along the abscissa: 1 = Right index finger tip, 2 = right second toe tip, 3 = volar surface of right forearm, 4 = middle of forehead, 5 = mid-epigastrium, 6 = anterior surface of the right thigh, 7 = posterior surface of the left thigh, 8 = right cheek, 9 = right axilla, 10 = left axilla, 11 = palm of the right hand, 12 = posterior surface of the right leg, 13 = right flank, 14 = plantar surface of the heel of the right foot, 15 = ball of the right foot, 16 = mid-plantar area of the right foot, 17 = lateral surface of the right arm.

Fig. 3. The mean and extreme values for the rate of water loss from 5 skin areas of 12 normal adults obtained during the months of July and August. * = variation in only one patient. The parts studied are represented by numbers along the abscissa and are the same as in figure 2.

(fig. 6). When the temperature is further increased only 5° and the relative humidity is kept at 75 percent there was a marked increase in the rate of water loss in all three parts. The measurements were not continued long enough to establish a new baseline.

Discussion. The water lost with the subject in a comfortable atmosphere was termed insensible, using the usual concept of terminology. The rate varied

considerably with the area of skin. In descending order it was as follows: hands, feet, head, arms, legs and trunk. The sensible water loss, stimulated by a hot and humid environment also varied with the area. The rate was greater for the finger tips, axillae and forehead. Although the insensible rate was slow from the skin of the trunk, the sensible rate became marked. The percentage increase was as great or greater from the skin of the arms, legs, and trunk, as from the finger and toe tips. The marked rate of sensible water loss from the

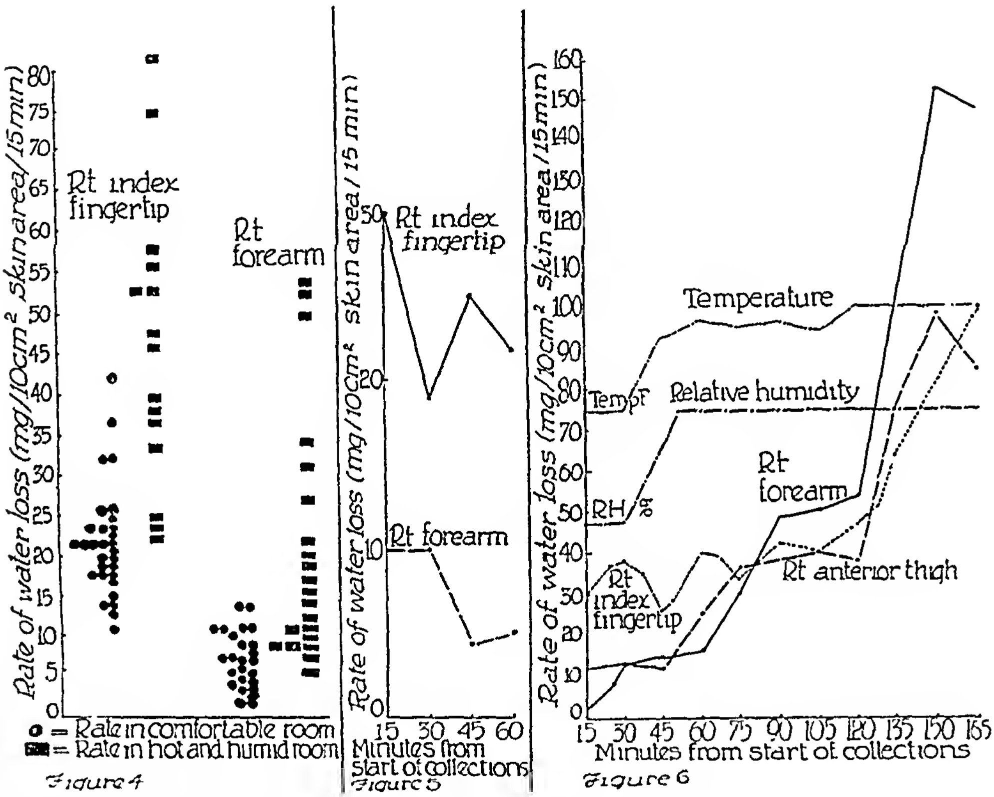

Fig. 4. Variations among individuals in the rate of water loss from two selected skin areas.

Fig. 5. Variations during one hour of the rate of water loss from two selected skin areas in one subject.

Fig. 6. The effect of variations of room temperature and humidity on the rate of water loss from three skin areas of one subject.

skin of the forehead and left axilla shown in figure 2 is due to the unusually rapid rate in one of the subjects studied. It is likely that if a larger number of subjects were studied such discrepancies would be smoothed out.

It would have been better to have measured all of these parts simultaneously rather than in groups of 3 or 4 parts at a time. The apparatus used in these studies was not constructed for so large a number. Kuno and his associates (2) used small celluloid chambers enclosing discs of blotter-paper to absorb the water that escaped to the surface of the skin. A larger number of these were

sealed on many different areas of the skin more or less simultaneously. The blotter-paper was weighed before and after absorbing sweat in order to determine the rate of water lost during a given time. Although such a method lends itself for easy measurement of many areas simultaneously, it is open to numerous errors and is relatively inaccurate. This probably accounts for the fact that the values found by Kuno and his associates are approximately half as great as the values found in these studies. Of course, the differences in race of the subjects, climatic conditions, and laboratory conditions (2) must have contributed to these differences. The relationships of the regional variations of water loss in these studies are essentially the same qualitatively as those reported by Kuno and his associates (2) and Galeotti and Macri (1).

The marked variations in the rate of sensible perspiration from subject to subject is not surprising (fig. 4). Everyone is acquainted with the variations of sweating in different individuals from his own casual inspection. Such marked variations in both insensible and sensible perspiration have been reported by many others (2). The factors that influence sweating that might be related to such variations were discussed in a previous report (5).

In a previous report (5) data indicated that there might be seasonal variations in insensible water loss from the skin measured under constant laboratory conditions. The present studies do not support such a contention. Since it is so difficult to control all factors that influence sweating so that the season is the only variable it would be preferable not to draw any conclusions from these or similar studies as yet.

As illustrated by figure 6, sensible perspiration in a resting adult does not become marked until the environmental temperature and humidity become very unfavorable for loss of heat from the body. When the room temperature was 95°F. and the relative humidity 75 percent and with the subject resting quietly in bed, the subject felt relatively comfortable although he knew he was in a very warm room. Sweating increased, but not markedly. These observations are in keeping with those of DuBois and his associates (6) who noted that under more or less similar conditions without muscular activity heat loss by radiation might be sufficient to maintain the normal thermal level of the body. When the room temperature was increased to 100°F. and the relative humidity maintained at 75 percent, there was an immediate and marked increase in the rate of sweating. Under such conditions heat was passing from the atmosphere to the body and convection currents (less than 20 ft. per min.) were of no value. The only possible source of heat loss from the body was through evaporation. This could not be marked because of the high relative humidity. Therefore, sensible perspiration which would be of relatively little value was stimulated and sweat poured. Such sweating, if prolonged, may be detrimental since it can disturb markedly the water and electrolyte balance. If such sweating continues over a period of a half to one hour or so the subject may show ill effects. At the temperature of 95°F. and a relative humidity of 75 percent and with the subject resting quietly, the rate of heat production and heat loss were about at equilibrium. Should production have increased as with mild exercise, body tempera-

ture would tend to rise and sweating result. When heat loss was interfered with by increasing the room temperature and humidity, sweating became marked even with the subject at rest. Under conditions of high temperature and humidity when heat loss cannot be rapid, such as happens in sub-tropical and tropical climates, exercise or heat production should be kept as low as possible.

SUMMARY

In a study of 17 different areas of skin of 46 normal adults it was found that there is a marked regional variation in the rate of sweating. The most rapid rates of insensible perspiration are from the hands, feet, forehead and cheeks. The skin of the trunk, arms and legs has relatively slow rates of insensible water loss. There are marked variations in the rate of insensible water loss for the same area from subject to subject and from time to time in the same subject. These marked variations result in overlapping of values for the various areas.

Similarly, there are marked variations in the rate of sensible perspiration stimulated by a hot and humid environment. The rate of water loss increased often to a greater extent from those areas which showed relatively little insensible sweating when sensible sweating occurred, than from the areas which showed the largest rates of insensible water loss.

There is no definite evidence of difference in the rate of insensible or sensible water loss during winter or summer months when the measurements are made under constant laboratory conditions.

The rate of water loss from the skin of a subject resting quietly in bed is not materially increased when the temperature and relative humidity are increased from 75°F. and 50 percent respectively to 95° and 75 percent. When the temperature is further increased to 100°F., sweat literally pours. In a humid sub-tropical and tropical environment, when heat loss is interfered with, muscular exertion should not be maintained for prolonged periods of time.

Acknowledgment. We wish to express our appreciation for the excellent technical assistance and keen interest of Mr. G. Morgavi, who participated in these studies and constructed the apparatus.

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EFFECTS OF INHALATION OF 100 PER CENT AND 14 PER CENT OXYGEN UPON RESPIRATION OF UNANESTHETIZED DOGS BEFORE AND AFTER CHEMORECEPTOR DENERVATION[1]

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Since the discovery of the aortic and carotid chemoreceptors, a large number of investigations have been performed upon these structures (for bibliography, see (13)). However there is still disagreement upon two points of fundamental importance. The first of these is the question of the existence of tonic activity. Some investigators (11, 8, 9) maintain that chemoreceptor reflexes are of great importance in the control of respiration under all conditions (normal as well as abnormal) while others (4) believe that these structures function chiefly as an emergency mechanism, of extreme importance during anoxemia, asphyxia, acidosis, and marked hypercapnia, but relatively unimportant in the control of normal breathing. The second point has to do with direct stimulation of the medullary centers by anoxia. Although experiments upon anesthetized animals indicate that anoxia usually stimulates the medullary centers only reflexly through the chemoreceptors, it has been reported (12) that anoxemia may stimulate the medullary centers directly if the anesthesia is sufficiently light. Since anesthesia may either intensify or depress chemoreceptor activity depending upon the nature and concentration of the anesthetic[3] we decided to investigate these problems on trained unanesthetized dogs.

METHODS. Mongrel female dogs of varying size and breed were trained to lie quietly upon a table while breathing through a moulded plaster mask reinforced with rubber and fitted with inspiratory and expiratory valves. All gases, including room air, were inhaled from identical Douglas bags attached to a 3-way stopcock on the inspiratory side. Minute volume of respiration was measured by passing the expired air through a gas meter and respiratory rate was measured by a pneumograph and tambour. The periods of inhalation of each gas were usually 6 minutes; when samples of arterial blood were drawn, these were col-

[1] This investigation was partly financed through the National Committee for Mental Hygiene from funds granted by the Committee on Research in Dementia Precox founded by the Supereme Council, 33° Scottish Rite, Northern Masonic Jurisdiction, U. S. A.

[2] Ellen Mickle Fellow of the University of Toronto.

[3] Chloralose (100 mgm./kilo intravenously) in the dogs used in these experiments depressed respiratory minute volume (average reduction 36 per cent) and arterial pO_2 (average reduction 25 mm. Hg) and raised arterial pCO_2 (average increase 5.2 mm. Hg). At the same time, the degree of anoxia and the amount of NaCN necessary to produce stimulation of respiration were lessened. Chloralose anesthesia therefore exaggerates the effects of chemoreceptor reflexes. Experience of other investigators (1) (6) (12) with different anesthetics has shown that the influence of anesthesia on chemoreceptor reflexes is too complicated to permit generalizations from experiments with one type and grade of anesthesia on one species of animal.

lected under oil from the femoral artery at the end of the 6 minute periods, heparinized, covered with melted paraffin and kept in ice until analyzed. Estimations of plasma pH were made with a closed glass electrode at 38°C. and CO_2 content and whole blood O_2 content and capacity were determined by the Van Slyke manometric method. Experiments performed during the summer months were carried out in an air-conditioned room at 70°F. A complete experiment consisted of a series of observations (inhalation of room air, 100 per cent O_2, room air, 14 per cent O_2, room air) until the responses were consistent. The same series of observations was repeated after denervation of the carotid and aortic bodies.

Carotid body denervations were done under ether anesthesia; the internal carotid and occipital arteries and all attached nerve tissue were divided between ligatures and the external carotids were stripped from the origin of the internal carotid to the origin of the lingual artery. Aortic body denervations were performed by Dr. Norman Freeman in the following manner: The dog was anesthetized by intratracheal insufflation of ether; through an incision in the right third intercostal space, all branches of the right vagus and recurrent laryngeal nerves to the heart and aorta were severed below the level of the origin of the latter nerve. All branches from the stellate ganglion to the right vagus were cut and one inch of this vagus was excised below the origin of the recurrent laryngeal. Great care was taken not to injure the right recurrent laryngeal nerve itself. Through a similar incision on the left side all branches of the left vagus inside the thorax were severed including the left recurrent laryngeal at its origin from the vagus, but the stripped left vagus trunk was left intact. This operation preserves one recurrent laryngeal nerve, one abdominal vagus trunk and a few of the fibers from the pressure receptors situated in the brachiocephalic artery, but interrupts all fibers from the aortic bodies. Complete chemoreceptor denervation was attested by the lack of respiratory stimulation from intravenous injections of NaCN or inhalation of low oxygen mixtures which previously had caused marked hyperpnea; incomplete aortic pressure receptor denervation was indicated by a return of blood pressures to the normal range within 2–4 weeks, after a temporary hypertension.

Results. A. *Tonic activity of chemoreceptors during quiet breathing of room air.* This was investigated by substituting 100 per cent O_2 for room air while respiratory rate and minute volume were measured. We chose this procedure because the arterial blood of an animal breathing room air always shows some oxygen unsaturation, and if this is sufficient to set up tonic chemoreceptor impulses capable of stimulating respiration, inhalation of 100 per cent O_2 should result in a definite depression of respiration. The average results of 194 observations on seven trained normal dogs are shown in figure 1. The scatterings of the findings in each dog are shown in table 1. In each dog there was usually an immediate decrease in minute volume which was maximal at the end of the first minute; the magnitude of this depression varied from 11 to 31 per cent. Minute volume had returned to a normal level by the end of 3 minutes in 3 dogs and by the end of 6 minutes in another, but in 3 dogs respiration was still 10

TABLE 1

Effect of inhalation of 100% O_2 upon minute volume of respiration

INTACT DOG	TOTAL OBSERVATIONS	DECREASE IN MINUTE VOLUME	INCREASE IN MINUTE VOLUME	NO CHANGE IN MINUTE VOLUME
1	44	35	9	
2	49	33	12	4
3	14	12	2	
4	12	12		
5	47	46	1	
6	13	12	1	
7	·15	15		
DENERVATED DOG				
1	6	2	4	
2	22	3	18	1
3	9	2	7	
4	6		5	1

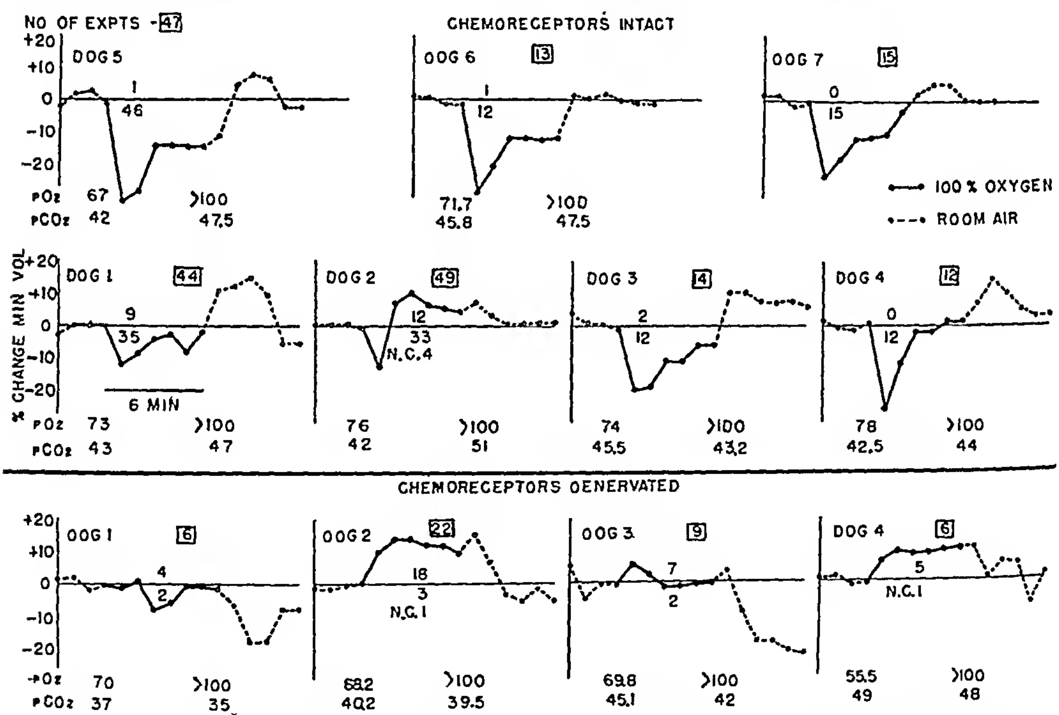

Fig. 1. Average effect of 100 per cent oxygen on respiration of the unanesthetized dog. Upper charts represent average percentage change in minute volume of respiration of normal unanesthetized dogs during inhalation of room air (broken lines), 100 per cent O_2 (solid lines) and then room air again; observations are recorded at minute intervals. The numbers in squares indicate the number of experiments performed upon each dog. The numbers just above the zero percent line represent the number of experiments in which respiratory minute volume increased, and those just below the line represent the number of experiments in which respiration decreased during inhalation of 100 per cent O_2 (N.C. = no change). Arterial oxygen tensions (pO_2) and arterial CO_2 tension (pCO_2) were determined upon femoral artery blood withdrawn at the ends of the control period and of the O_2 inhalation.

Lower charts show similar data upon dogs 1 to 4 after denervation of carotid and aortic bodies.

to 14 per cent below the control level at the end of the 6th minute of O_2 inhalation; in these, return to normal occurred promptly upon inhalation of room air. In 5 of 6 dogs in whom CO_2 tensions of arterial blood were measured, pCO_2 had increased 1.5 to 9 mm. (average 3.8 mm.) at the end of the O_2 inhalation.

The same procedures were repeated after chemoreceptor denervation in 4 of the 7 dogs (fig. 1 and table 1). (One dog died before operation, one died following carotid denervation and a third has not been operated upon as yet.) The immediate decrease in minute volume that occurred consistently when O_2 was inhaled by the intact dogs was lacking completely after the denervation.

Comment. Since all these animals usually showed an immediate depression of pulmonary ventilation when they were made to breathe 100 per cent O_2, and since chemoreceptor denervation entirely abolished this effect, it follows that some of their chemoreceptor units must have been tonically activated by the oxygen unsaturation normally existing in their arterial blood during quiet

TABLE 2

Arterial blood analyses—breathing room air

DOG	O_2 SAT.	pCO_2	pH	M.V. (AVERAGE)
1 Intact	95	43	7.40	2540
Denerv	95.5	37	7.48	2500
2 Intact	95.5	42	7.40	2350
Denerv	95.4	40.2	7.44	2700
3 Intact	94.0	45.5	7.37	2500
Denerv	94.2	45.1	7.38	2800
4 Intact	95.0	42.5	7.35	2550
Denerv	88.0	49.0	7.35	3400

breathing of room air at sea level. The interpretation of this finding, however, should be made with due regard for the following facts:

1. Denervation of the carotid and aortic bodies did not lead to a lower resting volume of pulmonary ventilation in any of these animals (table 2), or to any significant change in arterial gas content or pH except in dog 4, which we believe had some atelectasis. Hence it would appear that tonic chemoreceptor activity was not essential for the maintenance of normal respiratory activity in these animals breathing room air. The same conclusion is suggested by the tendency of breathing to recover during the inhalation of O_2 in 4 out of 7 intact dogs, and by the qualitative variations in the responses of the same animal from day to day. This is particularly evident in dogs 1 and 2 (table 1).

2. Inhalation of 100 per cent O_2 at sea level should cause a rise in arterial pO_2 from a normal of 70 to 90 mm. Hg to something above 600 mm. Hg, which is of course far beyond the physiological range. When these dogs were made to breathe mixtures low in O_2 we obtained evidence, confirmatory of a previous report (4), indicating that a change of 30 mm. Hg in arterial pO_2 (i.e., from 80 to

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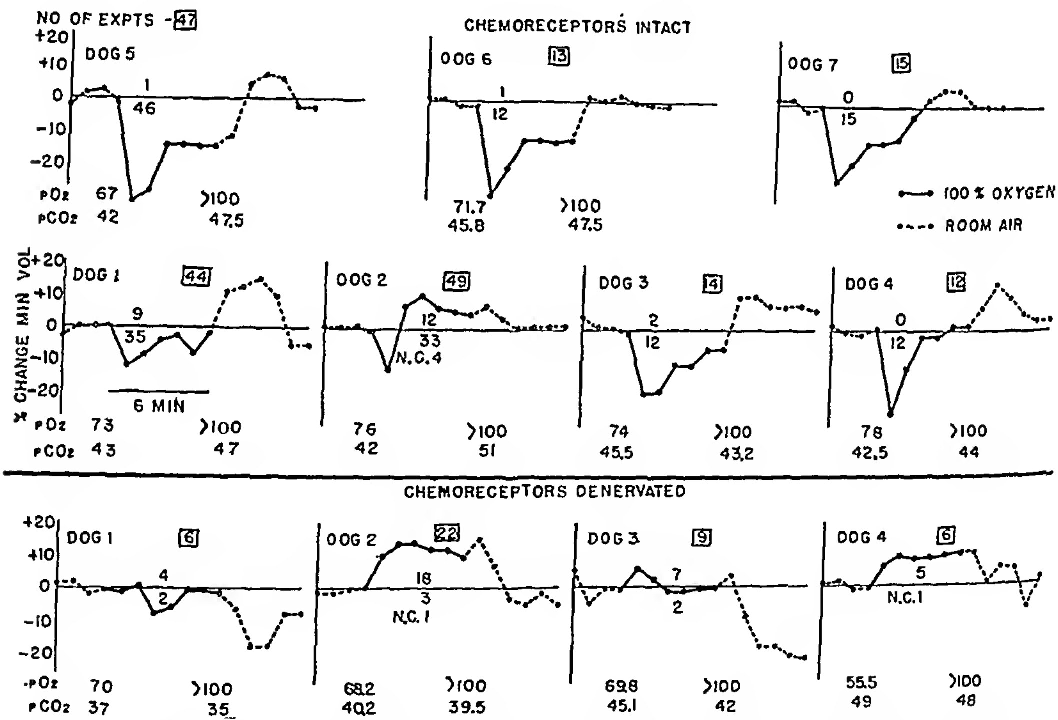

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50 and back to 80 mm. Hg) causes practically no change in respiratory minute volume in the dog, but a further change of 10 mm. Hg (i.e., from 80 to 40 mm. Hg) causes definite hyperpnea and the reverse change (from 40 to 80 mm.) leads to abrupt depression of breathing. At the critical level, therefore, small changes in arterial pO_2 cause more marked alterations in chemoreceptor activity than do much larger changes in pO_2 at a normal or supernormal level. This supports the suggestion (13) that while some chemoreceptors are sensitive enough to be activated by the small degree of arterial unsaturation normally present, most of them come into action only at a definitely subnormal pO_2.

3. Even though some chemoreceptors show tonic activity in dogs at sea level, they do not appear to do so in man. It has been reported (15) that O_2 inhalation causes respiratory stimulation in healthy young adults, but the conclusion was based on the average obtained during a 15 minute period and no mention is made of the immediate effect. We investigated this point in 11 young adults. Of 19 experiments, the immediate effect of inhaling 100 per cent O_2 instead of room air was an increase in respiratory minute volume in 13, a decrease in 4, and no change in 2. The average of the 19 observations gave a 6 per cent increase. These findings suggest that tonically active, oxygen-sensitive chemoreceptors are the exception rather than the rule in normal man.

While the existence of chemoreceptors tonically activated by the degree of oxygen unsaturation normally present in unanesthetized dogs is definitely confirmed, no evidence was obtained bearing upon the question of similar activation by changes in CO_2, pH, or temperature (8, 14).

B. *Direct effects of anoxia on the respiratory center.* The same dogs were given 14 per cent O_2 to inhale for a 6 minute period using the same experimental technique described in A. The average results, before and after denervation, are shown in figure 2. In each of the 7 normal dogs, 14 per cent O_2 increased respiratory minute volume, the range being 17 to 29 per cent; in the 4 denervated dogs the initial effect was depression of respiration by 22 to 29 per cent. These results are similar to those reported in unanesthetized (2) and in anesthetized dogs (7). The absence of stimulation in the denervated dogs cannot be attributed to a failure of arterial pO_2 to fall, for the average arterial pO_2 (calculated according to Dill's data (5)) during inhalation of 14 per cent O_2 was 48 mm. Hg before denervation, 33 mm. Hg afterward. The first effect of anoxia upon respiration of these dogs was unquestionably depression because rate, depth of breathing, and respiratory minute volume were decreased in each of the 4 dogs and in every one of the 25 observations made with 14 per cent O_2 after the denervation. The depression began within the first minute and lasted about four minutes, after which the minute volume began to increase, though it was still 12 to 20 per cent below normal at the end of the six minute inhalation period. This apparent recovery was associated with restlessness and in several instances convulsive movements were observed, but it is noteworthy that the increase in breathing was due entirely to acceleration, often of the rapid, shallow type; depth was never increased and was usually decreased.

Comment. These findings are presented as additional evidence that the char-

acteristic respiratory response to anoxemia (prompt increase in depth of breathing causing a diminution in the ΔpO_2 between inspired air and arterial blood) is due to chemoreceptor reflexes. This conclusion is not vitiated by the fact that, in the denervated dog exposed to atmospheres low in oxygen, the primary respiratory depression may be followed by an increase in respiratory minute volume toward or above normal (2) (12). While we have not prolonged the low O_2 inhalations in our experiments beyond 6 minutes, we did notice, following the depressed period of 4 minutes, an unmistakable tendency for the minute volume to rise despite continued anoxia; this was due entirely to an increase in rate, depth being unaffected or decreased. Goldschmidt, Brewer, Daven-

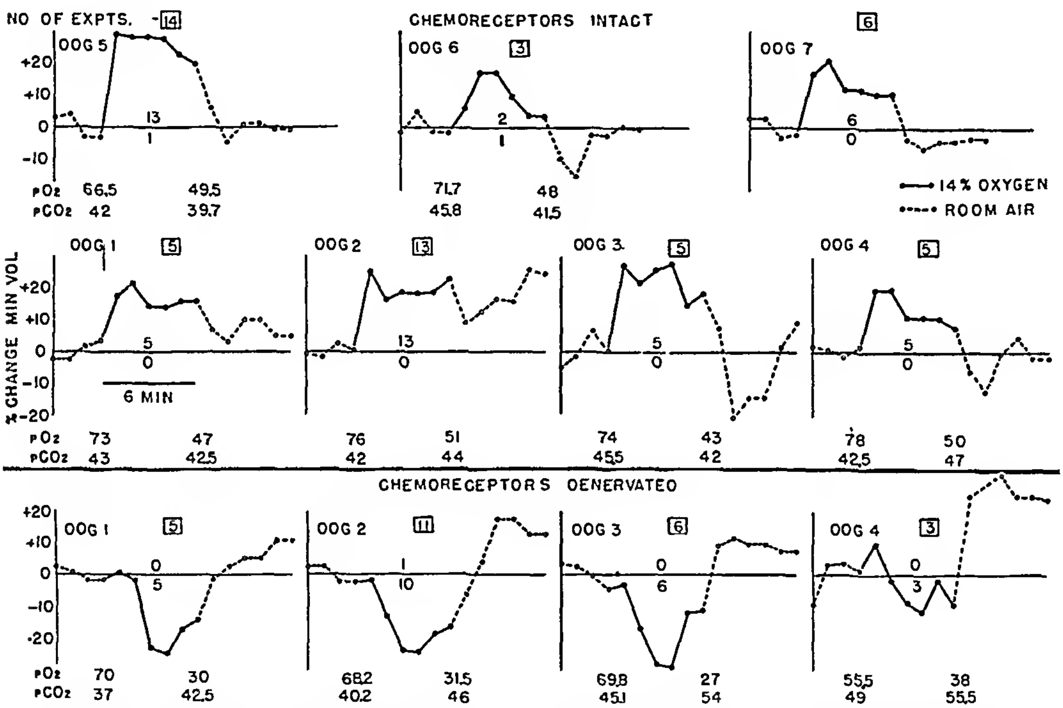

Fig. 2. Average effect of 14 per cent oxygen on respiration of the unanesthetized dog. Similar to figure 1 except that solid lines represent inhalation of 14 per cent O_2 in 86 per cent N_2.

port and Chambers (10) have analyzed the effects of prolonged anoxia in the surviving 3 of our 4 denervated dogs, and have found that minute volume eventually returned to normal and then usually rose above normal.

Several explanations might be offered for this delayed response other than regeneration of nerve fibers: *1.* Chemoreceptors elsewhere (e.g., in the coccygeal body) possessing much lower sensitivity to anoxia than those in the carotid and aortic bodies might be responsible; this is unlikely because this particular response appears to be depressed easily by anesthesia (12) while the carotid and aortic chemoreceptors are characterized by a high resistance to most forms of anesthesia (4).

2. As the oxygen saturation falls to lower levels upon prolonged exposure to

low oxygen, acid metabolites may accumulate in the respiratory center and so stimulate respiration. While there is no direct evidence favoring this explanation, it is in harmony with the views of those physiologists (9) who believe that respiration is controlled predominantly by the hydrogen ion concentration within the cells of the respiratory center. Since anoxia of this degree probably would result in accumulation of greater amounts of acid than under ordinary conditions, it might be pertinent to inquire why acidity in this instance increases only the rate of breathing, and why a latent period of 4 minutes must elapse before stimulant concentrations are reached. If acidity in general (rather than CO_2 specifically) is the characteristic stimulus to the respiratory center, it should act promptly and should affect both rate and depth, for excess CO_2 is known to increase both rate and depth very promptly whether given by inhalation or by direct injection into the respiratory center (3). Since in the case of anoxia the acid metabolites are formed presumably within the cells of the center, the question of relative rates of diffusion of acid and CO_2 should be irrelevant. It may be argued that anoxia by a dual mechanism is depressing while stimulating, and consequently the acid-mechanism is working at a disadvantage. However anoxia does not correspondingly alter the response of the center to CO_2 inhalation. Dumke, Chiodi and Schmidt (7) found in denervated dogs average increases in respiratory minute volume of 52 per cent during inhalation of 3.5 per cent CO_2 in O_2, and of 46.4 per cent on inhalation of the same CO_2 concentration in 10 to 12 per cent O_2; in all cases, both rate and depth were increased and the hyperpnea appeared promptly. If CO_2 is effective only by virtue of its acid properties, it would be rather remarkable that the response to it is not markedly reduced by a degree of anoxia that drastically alters the response to a direct increase in intracellular acidity.

3. A third explanation is suggested by the observations that this polypnea is abolished by decerebration (2) or by a slight increase in the depth of anesthesia (12), which also suggests a supra-tentorial origin. However, preliminary experiments upon decerebrated cats and dogs have occasionally shown acceleration of rate after an initial depression of depth and minute volume when 14 per cent O_2 was breathed after carotid denervation and vagotomy. Consequently the phenomenon is not necessarily dependent upon the higher centers.

CONCLUSIONS

In 7 unanesthetized dogs, inhalation of 100 per cent oxygen for 6 minutes led to a transient diminution in respiratory minute volume varying from -11 to -31 per cent. After denervation of the carotid and aortic bodies, oxygen inhalation produced no change or an increase in minute volume of respiration. Consequently some chemoreceptors in the dog must be continually activated by the usual degree of oxygen unsaturation of arterial blood at sea level. However experiments upon normal men failed to reveal evidence of similar tonic activity.

Inhalation of 14 per cent O_2 in unanesthetized dogs increased respiratory minute volume 17 to 29 per cent; after chemoreceptor denervation, initial depression of minute volume (22–29 per cent) was observed. However, unlike the

sequence in anesthetized dogs, in which respiratory depression is usually progressive until death, in the unanesthetized denervated dogs depression of depth and rate of breathing was succeeded by acceleration of rate. Therefore known chemoreceptor reflexes cannot be responsible for all the increase in rate during prolonged anoxia. The possible causes of this delayed response are discussed.

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A STUDY OF THE EFFECT OF SPONTANEOUS VARIATIONS IN BLOOD PRESSURE UPON SPONTANEOUS VARIATIONS IN THE VOLUME OF THE FINGER TIP[1]

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During the past few years reports from Burton (1), Hertzmann and Dillon (2) and Burch, Cohn and Neumann (3) have established the fact that small blood vessels undergo spontaneous variations in volume not related to any recognized effect of respiration or heart beat upon the peripheral circulation. Burch, Cohn and Neumann have described in detail some of these fluctuations as they occur in fingers, toes and pinnae. Records made with a pneumoplethysmographic technique showed rhythmic changes in volume which were classified in three general types and named alpha, beta and gamma waves. The alpha waves, representing the most rapidly recurring fluctuations had an average rate of 7.9 deflections per minute and average size of 14.5 cu. mm. per 5 cc. of finger tip.[2] The beta and gamma waves were larger and slower than the alpha waves and represented gradual changes whereas, in most instances, alpha deflections were completed within a few seconds. It is to be emphasized that none of these changes were regular in frequency or volume; each in turn, including the effects due to cardiac impulse and respiration, was imposed upon the next larger and slower "rhythm" to give a continuously changing record of the size of the part studied.

With the origin of these waves in doubt, one of the problems which arises concerns itself with their dependence upon the blood pressure. Steele (4) in his study of intra-arterial measurements of the blood pressure in human subjects noticed variations both of systolic and of diastolic pressures, independent of respiration and raised the question of relationship between fluctuations in pressure and those in the volume of peripheral parts. To settle this question simultaneous records have been made of the intra-arterial blood pressure and of alpha waves.

Materials and method. The apparatus consists of two important parts, of which one is the sensitive pneumoplethysmograph of Turner (5) and the other the hypodermic manometer of Hamilton, Brewer and Brotman (6). Both these devices, together with the necessary camera and timers, were assembled in a single unit which could easily be transported to the bedside. All of the working parts were enclosed in a light-tight case to permit the subject to be in a lighted room at the time of the study.

The hypodermic manometer was built in accordance with the design of Hamil-

[1] This is the tenth paper reporting the results of studies of the small blood vessels and related subjects.

[2] In this paper, changes in volume are given in cubic millimeters per 5 cc. of part.

ton. The necessary changes to adapt the apparatus to the purposes of the present investigation were limited to: 1, the use of a prism and a mirror to shorten the over-all length of the light beam and yet retain a desirable degree of sensitivity with minimum movement of the membrane, and 2, the use of a simple dampening device, a heavy cube of rubber four inches square attached to the bracket supporting the manometer, to decrease the vibrations occasioned by the motor driving the camera which is part of the assembly. The mirror used to reflect light from the membrane of the manometer had a focal distance of six feet. By reflecting the light twice, first 180° by a prism and second, another

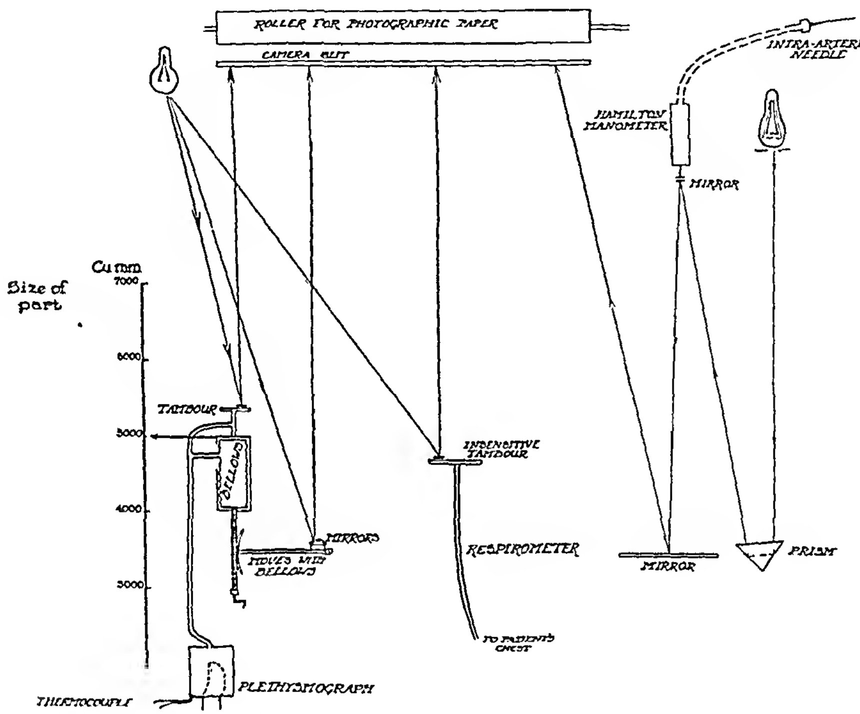

Fig. 1. Schematic drawing is reproduced of the arrangement of the various parts of the apparatus. The bellows and tambour of the plethysmograph are moved proportionately nearer the camera when records are being made from large finger tips and farther away when small finger tips are used. The angles of reflection of light are markedly exaggerated.

180° from the mirror of the membrane, it was possible to keep the total length of the apparatus within four feet (fig. 1). The Hamilton manometer and its base line mirror were mounted on a swivel stand which was firmly screwed to the baseboard of the apparatus.

The fluid used in the eighteen-inch flexible lead tube connecting the intra-arterial needle with the manometer was physiological saline solution to which were added merthiolate to a concentration of 0.01 per cent and sodium citrate to a concentration of 1 per cent. The fluid remained sterile both on aerobic and anaerobic culture. As an additional precaution against infection, the tubing was thoroughly flushed with a fresh supply of this fluid just preceding each test.

The plethysmograph of Turner was modified slightly to allow a simpler method of comparison among records of the subjects. Instead of a fixed distance between the tambour of the plethysmographic system and the camera, the system was movable on a sliding mount. The distance between tambour and camera was lengthened and shortened depending upon whether the finger tip was respectively smaller or larger than the average volume of 5 cc. The volume of the finger tip was ascertained for each subject and the apparatus adjusted accordingly. Once a tambour of desirable sensitivity was secured, the records obtained with its use were comparable for all subjects; no further calculations were necessary.

The subjects used for this investigation included four normal young adults, four adults with hypertension, and two convalescent patients free from circulatory disease. They varied in age from 20 to 40 years. Two of the normal subjects were female, all of the others were male. An attempt was made to acquaint all of these volunteers with the nature of the problem and the technique employed, by making a series of preliminary records of the volume changes of the finger tip during the week or two preceding the simultaneous recording of intra-arterial blood pressure and of fluctuations in volume of the finger tip. Previous studies (7) have shown the importance of eliminating, in so far as is possible, the distracting features of a laboratory in order to decrease the influence of extraneous stimuli upon spontaneous physiological variations. The attempt to do this during the present investigation was less than fully successful because of the necessity for having at least one observer present during each test.

The left index finger was selected for recording changes in volume and the left radial artery at the wrist for obtaining simultaneous records of blood pressure. After the subjects had been allowed to rest for a half-hour an air-tight cup was sealed to the tip of the left index finger and connected to the tambour of the plethysmograph. With only one observer, well known to the subject in the room, a record of the spontaneous changes in the volume of the finger tip (alpha waves) was made usually for a period of a half-hour. A second observer then entered the room, quickly made a small intradermal wheal with 1 per cent novocaine solution over the site of injection and inserted the needle (gauge 23) into the left radial artery. Simultaneous records were made for variable periods of time, the longest being eleven minutes and the average five minutes. Half of the subjects said that they experienced momentary pain shooting upwards towards the shoulder at the time of insertion of the needle. The pain subsided rapidly and was not present at the time the records were made.

Results. Close comparison of the alpha deflections of the finger tip with the spontaneous variations in blood pressure established the fact that they were independent of each other though their average frequencies were almost the same (about 6 times a minute). In addition, some changes in alpha deflections as large as 50 cu. mm. in the course of a minute occurred without any preceding, simultaneous or subsequent, measurable change in blood pressure. In a previous communication (3) it was reported that alpha deflections recorded simultaneously from the tips of the index fingers of opposite hands were usually but

not continuously concordant. This possibility was demonstrated again during the measurement of intra-arterial blood pressure in one subject from whom deflections were recorded of both the left and right sides. As can be seen from the illustration (fig. 2) the volumes of these varied discordantly for part of the time and neither bore any relationship to the essentially constant level of blood pressure.

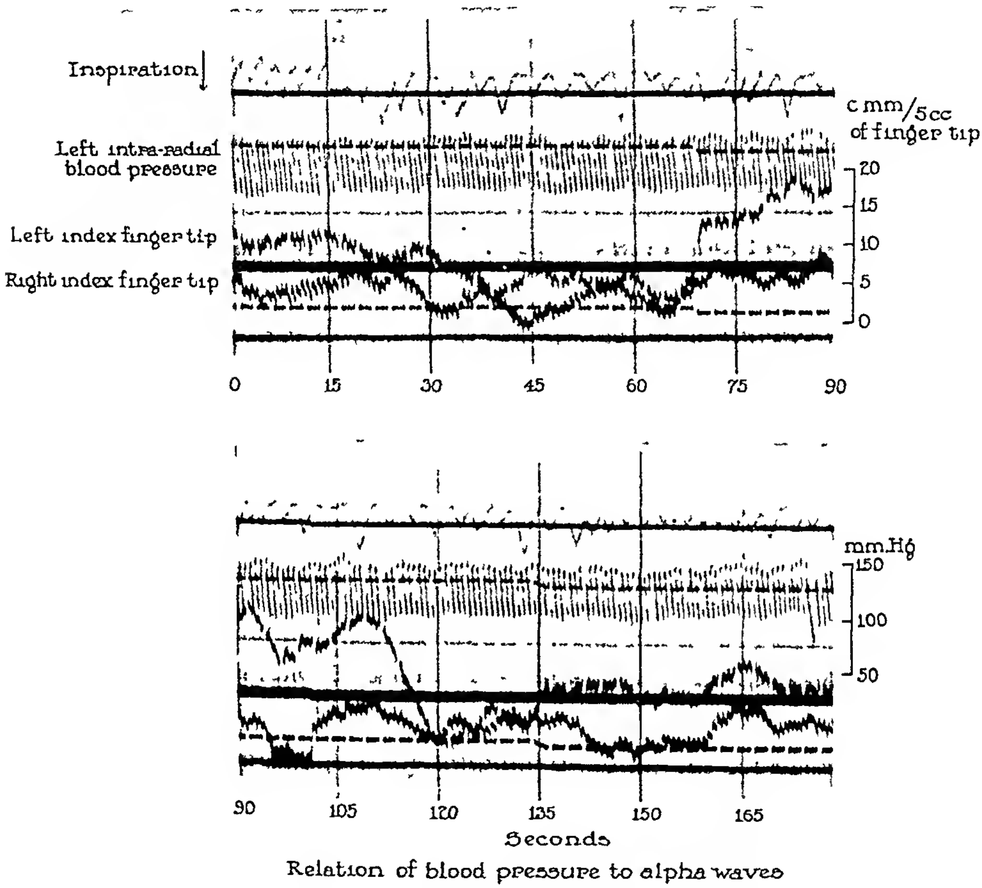

Fig. 2. A comparison between intra-arterial blood pressure and alpha deflections is shown. Alpha deflections of the two index finger tips occur independently of each other and without reference to changes in blood pressure. The record of the tip of the left index finger is the one which is interrupted by the white lines at regular intervals.

The records of intra-arterial blood pressure showed two types of rhythmic fluctuation, occurring both in systole and diastole. One occurred simultaneously with respiration. Expiration was consistently accompanied by a slight rise (2 to 5 mm. Hg). This phenomenon has recently been studied in dogs by Hamilton, Woodbury and Vogt (8) who ascribed it partly to an increase in intra-thoracic pressure and partly to an increase in cardiac output.

The other type was not so constant. Four of the ten subjects (2 normal adults, 2 hypertensive patients) showed rhythmic increase and decrease in blood pres-

sure, occurring four to six times a minute and involving a systolic change of 15 to 30 mm. Hg and a diastolic change of 10 to 20 mm. Hg (fig. 3). Although these oscillations had the frequency of Traube-Hering waves, they occurred in human subjects who were awake; classical Traube-Hering waves have been described only in morphinized or curarized animals. Steele recently has obtained similar fluctuations while using a comparable technique (4).

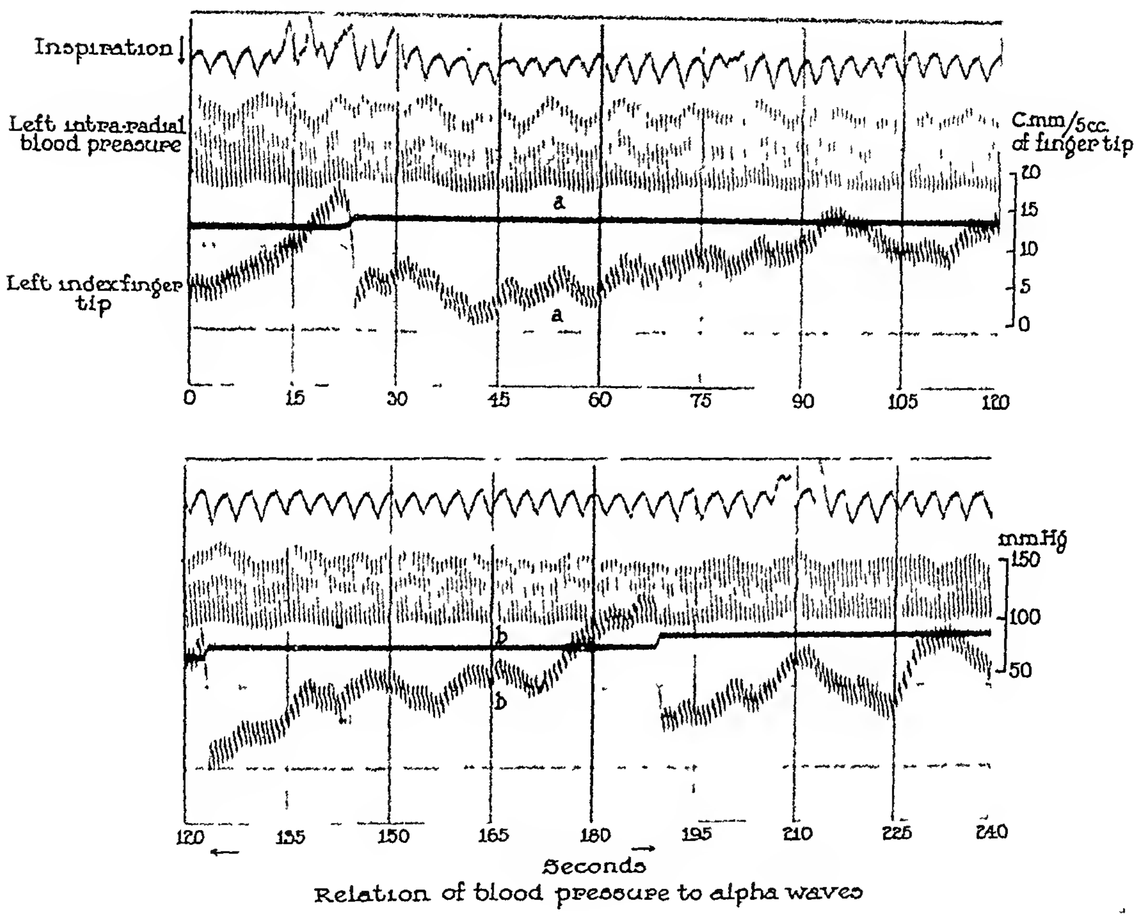

Fig. 3. Another comparison between an intra-arterial blood pressure record and alpha deflections of the index finger tip is shown. In the portion of the record marked *a*, spontaneous variations in blood pressure move in the same direction as small alpha deflections. This is an uncommon picture. The more usual occurrence is shown at *b* where alpha deflections are completely unrelated to changes in blood pressure.

Spontaneous variations in volume of the finger tip were recorded in all subjects, corresponding in frequency and size with the results previously reported (3). Before the insertion of the intra-arterial needle the size of the alpha and pulse waves was larger than after this was done. The change occurred as soon as each subject realized that the needle was about to be inserted but not at the moment of touching or piercing the skin or arterial wall although further vasoconstriction developed then. No attempt was made to measure the exact

sizes either of the alpha or pulse waves because they were obtained during a period of subjective anxiety and tension apparently related to the punctures. All the subjects admitted some anxiety at the prospect of being stuck with a needle. But this was not enough to prevent the few, in whom the introduction of the needle was not immediately successful, from volunteering a second time. A second attempt after an interval of a few days was better than continued probing. The importance of anxiety as a modifier of the size of alpha deflections corresponded with other experiences (9).

In the record of one subject a difference from the usual complete dissociation between alpha deflections and variations in blood pressure was noticed (fig. 3). During the course of approximately one minute, spontaneous decreases or increases in blood pressure (systolic and diastolic) were respectively concordant with small decreases or increases in the volume of the finger tip (fig. 3a). No other portion of this record showed similar agreement. It is significant, perhaps, that no large alpha deflections were in progress at the time. The meaning of such fleeting relationships is not known, but it would appear that a series of very small changes in volume may require to be interpreted as the effect of changes in blood pressure.

In those records illustrating a prominent respiratory influence both upon the alpha waves and upon the blood pressure, the changes occurred simultaneously. Inspiration was then associated with small decreases both in blood pressure and in the size of the finger tip. Whenever the subject momentarily held his breath, both types of response disappeared, only to return with the onset of respiration. A similar type of concordance occurred immediately following pauses in cardiac action (skipped beats). In such instances when the blood pressure fell, the direction of the alpha deflection was downward until the next heart beat occurred. Then the blood pressure was immediately restored to its original level (within one or two beats) but a delay of about 15 seconds intervened before the volume of the finger tip was completely regained. In this period of recovery, the volume of the finger tip gradually increased while the blood pressure was maintained at a constant level.

Discussion. Although it has been shown that variations in volume of the finger tip (alpha deflections) are independent of normal spontaneous changes in blood pressure, the circumstances under which this investigation was carried out actually made the demonstration of this independence difficult. The average size of alpha deflections depends in great part upon the degree of relaxation of the subject at the time the record is made (9). When anxiety and tension are prominent, alpha deflections tend to be small. Throughout the period of simultaneous recording of blood pressure and alpha waves, the latter were smaller than before the insertion of the needle into the artery. Had it been possible to obtain the records of blood pressure without the introduction of a needle, there would have been less cause for anxiety on the part of the subject. Alpha deflections would then have been larger and the lack of correlation between alpha deflections and changes in blood pressure even more impressive. It must be emphasized that the change from large alpha deflections obtainable be-

fore to smaller ones afterward did not occur at the moment of penetration into the arterial wall, but when the patient was first aware that the needle was to be inserted. This point is emphasized in order to exclude the possibility that the change in size of alpha deflections resulted from a reflex initiated by irritation of the vessel well.

The fact that alpha deflections are independent of changes in blood pressure is supported by the frequency with which records obtained simultaneously from the finger tips of opposite hands show discordant deflections. This occurs in about 25 per cent of the number of deflections (3). Similar discordance has been noticed when simultaneous records are made from the index finger and second toe. As a matter of fact, the difference in time (0.3 sec.) between constriction of a finger tip and that of a toe tip following the application of an external stimulus such as light or heat upon a distant part of the body is far greater than might be expected if such responses resulted primarily from a change in systemic blood pressure (fluid transmission) and approximates that to be expected from the transmission of nerve impulses to constricting elements in fingers and toes (10).

One of the main reasons for studying the relationship of spontaneous variations in blood pressure to the occurrence of alpha deflections was the hope that a better understanding of the mechanism underlying alpha deflections would evolve. But, in this regard, the evidence obtained was only of negative value. But upon what alpha deflections depend and what is the rôle of such changes in the economy of the body are still unsolved problems. Certainly all superficial tissues do not exhibit such fluctuations, at least not of the magnitude discovered in fingers and toes. Hertzmann and Roth have recently emphasized this point anew (11).

This difference in behavior in different parts of the body is not limited to variations in volume, but has also been noticed in the loss of water from the skin. This, like the activity of blood vessels, is under the control of the sympathetic nervous system. The rate of water loss from fingers and toes, for example, is greatly in excess of that from forearm or chest during rest in a comfortable evnironment and undergoes spontaneous variations which are as impressive as are changes in volume (12, and unpublished data). An inquiry into the nature of these phenomena is not answered by making this comparison but it suggests that alpha deflections are not isolated physiological oddities.

Chambers (13) has observed alternating constriction and dilatation of individual arterioles. The observations were made in rats, but it is a near step toward regarding alpha deflections in human fingers as comparable phenomena. If the constriction and dilatation of arterioles are mechanisms which provide for the exchange of tissue fluids, then the study of alpha deflections assumes a new importance in understanding the transport of fluids in the body.

SUMMARY

By the simultaneous use of a plethysmograph for recording changes in volume of the tip of the left index finger and of an intra-arterial manometer for obtaining

synchronous readings of the blood pressure of the left radial artery, it was shown that the spontaneous variations (increase or decrease) in volume of the finger tip are not concordant with spontaneous changes in blood pressure (? Traube-Hering waves) and are present even in the absence of measurable variations in blood pressure. A few exceptions were noticed. Rises in systemic blood pressure during expiration were accompanied by variable but small increases in volume of the finger tip. Marked lowering of blood pressure accompanying cardiac asystole was reflected in a decrease in volume. The rule then seems to be that variations in the volume of the finger tip usually go on independently of changes or lack of change in blood pressure though under certain conditions there may be a transitory relationship. When present, it is manifested by an increase in volume when there is a rise in blood pressure.

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VARIABILITY OF CERTAIN FACTORS IN THE BLOOD PICTURE OF WOMEN[1,2]

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The usual criterion for determining the severity of an anemia is the extent of the divergence of the individual's blood picture from a range of values which have been established from measurements on many persons of the same sex and age. This range represents the difference between individuals and gives no indication of the variability that may be expected in the same person who is the subject of repeated observations. The latter type of information would be of value in interpreting clinical findings on subjects whose progress toward recovery is being followed by successive measurements. Studies reported in the literature dealing with the intra-individual variability have included relatively small numbers of subjects and few blood factors.

Platt and Freeman (4) made monthly hemoglobin determinations over a period of six months on 29 children ranging in age from 21 to 44 months and noted a maximum difference in the mean values of 2.9 grams per 100 ml. of blood in that interval of time. Weekly determinations of the hemoglobin values of 30 young medical students, men and women, were made by Ingersoll (1) for a period extending from October to January. The difference between maximum and minimum values varied for the different subjects from 0.4 to 2.7 grams per 100 ml. of blood, with the women showing greater differences than the men. A further contribution to this field is the study by Jellinek (2) who investigated the intra-individual variability in erythrocytes and leucocytes in 30 normal men, as well as a group of schizophrenics. He found a significant increase in the intra-individual variance in both erythrocytes and leucocytes with increasing time-intervals between observations. He noted further that the individual is significantly more homogeneous than the group with respect to both these factors.

The present study reports the results secured on a large number of healthy college women on whom observations were made at intervals varying from 1 week to 6 months. Two or more observations were made on each subject. In addition, the results of a series of day-to-day determinations on four women over periods of from 27 to 39 days are recorded.

Procedure. In most cases blood samples were secured by finger-tip puncture

[1] Approved for publication by the Advisory Committee as paper no. 18 of the Regional Project of the North Central States Relating to the Nutritional Status of College Women.

[2] Paper 2035 Scientific Journal Series, Minnesota Agricultural Experiment Station. Journal Paper No. J1051 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 538.

between 8 and 9 a.m. from subjects in basal condition.[3] Hemoglobin values were determined on duplicate samples by the Newcomer method using a disk which had been standardized by the oxygen-capacity method. Red and white cell counts were each made on two separate dilutions of blood using Thoma automatic pipettes, certified by the United States Bureau of Standards. Packed cell volume was measured by means of Van Allen hematocrit tubes, with heparin as the anti-coagulant. Duplicate samples were centrifuged for 30 minutes at

TABLE 1

Intra-individual variations in the blood picture of women, based on periodic measurements

TIME INTERVAL	NUMBER OF CASES	MEAN OF INITIAL READINGS	MEAN OF FINAL READINGS	MEAN DIFFERENCE	STANDARD ERROR OF A DIFFERENCE
Erythrocytes (millions per c.mm.)					
Within one week	227	4.25	4.25	0.00	0.20
Within two to six weeks	111	4.25	4.23	−0.02	0.29
Within seven weeks to six months	53	4.40	4.52	0.12	0.30
Hemoglobin (grams per 100 ml.)					
Within one week	238	13.10	13.05	−0.05	0.79
Within two to six weeks	137	12.96	12.89	−0.07	0.87
Within seven weeks to six months	92	13.57	13.68	0.11	1.20
Packed cell volume (per cent)					
Within one week	229	39.30	39.06	−0.24	1.63
Within two to six weeks	112	38.93	38.76	−0.17	1.72
Within seven weeks to six months	51	39.14	39.51	0.37	2.35
Leucocytes (thousands per c.mm.)					
Within one week	217	6.16	6.09	−0.07	1.26
Within two to six weeks	93	6.00	5.99	−0.01	1.72
Within seven weeks to six months	33	6.16	6.05	−0.11	1.53
Erythrocyte diameter (microns)					
Within one week	68	7.38	7.36	−0.02	0.11
Within two to six weeks	36	7.28	7.26	−0.02	0.15
Within seven weeks to six months	37	7.22	7.34	0.12	0.20

2750 revolutions per minute. For red cell diameter measurements, dilutions were prepared with Hayem's solution, one diameter of 200 round cells being measured immediately by means of a calibrated filar micrometer.

Results and discussion. In table 1 is shown the intra-individual variation in the blood picture of healthy young women which occurs when observations are made within one week, within two to six weeks, and within seven weeks to six months. Scrutiny of the table reveals that the mean difference between the

[3] One hundred of the samples were secured between 8 a.m. and 12 noon following a light breakfast.

initial and final values for the various factors is very small. The standard error of a difference, which is a measure of variability and defines the limits above and below the mean within which approximately two-thirds of the cases may be expected to fall, increases sharply with extension of the time-interval between the determinations. This observation is in agreement with the previously cited finding of Jellinek.

As an indication of the variation that may be anticipated in an individual blood picture during a limited period of time, a more intensive study on four individuals was made. The means and standard errors of the day-to-day values were computed and are given in table 2. In a normal individual the variation in blood values is influenced not only by true physiological alterations but also by the inaccuracies that are inherent in the methods employed. Since the measurements in the present study were made by skilled workers, the changes noted are such as may be expected clinically in consecutive observations of these blood factors on the same subject. It will be noted that the variability in the

TABLE 2

Intra-individual variations in the blood picture of women, based on day-to-day measurements

SUBJECT	DAYS OF OBSERVATION	ERYTHROCYTES		HEMOGLOBIN		PACKED CELL VOLUME		ERYTHROCYTE DIAMETER	
		Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error
		millions/c.mm.	*millions/c.mm.*	*grams/100 ml.*	*grams/100 ml.*	*per cent*	*per cent*	*microns*	*microns*
1	27	4.57	0.10	13.94	0.81	38.04	1.76	7.18	0.18
2	28	4.49	0.18	13.63	0.54	39.59	1.62	7.13	0.10
3	39	4.00	0.14	11.53	0.69	36.61	2.00		
4	39	3.94	0.14	12.13	0.71	38.20	1.26		

blood data of these four subjects was no greater than might have been expected when the variance obtained for the larger group of subjects in the two to six weeks time-interval is used as a criterion (table 1). In those few instances in which the data were somewhat more variable the differences were not great.

A number of workers have investigated the effect of menstruation on the blood picture of women (Smith and McDowell, 7; Reich and Green, 5; Smith, 6; and Leverton and Roberts, 3). These workers report that fluctuations occurred in the blood factors studied during the menstrual cycle irrespective of the phase of the cycle in which the observations were made. Although Ingersoll (1) reported that the women in her study manifested a greater intra-individual variability in hemoglobin than did the men, a comparison of the variances in erythrocyte and leucocyte counts within one week for the women subjects in the current study, 0.20 million and 1.26 thousands, respectively, with those reported by Jellinek for 30 normal men, 0.27 million and 1.76 thousands, respectively, shows a somewhat lesser variability for the women than for the men. However, due to the wide difference in the number of cases studied no conclusion can be drawn as to the comparative variability of the two sexes.

SUMMARY

Erythrocyte and leucocyte counts, hemoglobin and packed cell volume determinations, and erythrocyte diameter measurements were made on a large group of healthy young women at intervals varying from one week to six months. The standard error of a difference between the initial and final value was computed for each factor for three time-intervals, i.e., within one week, within two to six weeks, and within seven weeks to six months. These showed that the intra-individual variability increased with extension of time between measurements.

Day-to-day determinations on four subjects for periods ranging from 27 to 39 days indicated that the variability in the blood factors for these subjects was in agreement with that found for the larger group.

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ROENTGENKYMOGRAPHIC DETERMINATION OF CARDIAC OUTPUT IN SYNCOPE INDUCED BY GRAVITY[1]

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A common type of syncope is that which occurs after prolonged, quiet standing. Some individuals can maintain the upright posture for relatively long periods of time, whereas others show a tendency to faint soon after assuming the position. A subject may tolerate relatively long periods of quiet standing on certain occasions but not on others. Collapse, when it occurs, is assumed to be due to cerebral anemia consequent to a diminished venous return, resulting from excessive pooling in the capillaries and veins of the sub-cardiac tissues. The quantitative demonstration of the decrease in cardiac output under these conditions has been difficult. Thus in a previous investigation (1) in which the acetylene method was used, we were unable to find any greater decrease in cardiac output in fainters on prolonged standing than in non-fainters. However, the time required for the measurement of the cardiac output by this method and the necessity of obtaining the full co-operation of the subject precluded its use in the period just before collapse occurred. This was particularly true in those cases in which the onset of syncope was precipitate and rapid (see also 2, 3).

Two methods for the calculation of cardiac output which have been recently introduced seem better adapted to the problem: the ballistographic method of Starr and Rawson (4) and the roentgenkymographic method of Keys and his associates (5). Both are rapid and require a minimum of co-operation on the part of the subject. The vertical ballistocardiograph has been used by Starr and Rawson for studying the changes in cardiac output on arising. These investigators were unable, however, to use individuals subject to fainting because of uncontrollable muscular movements which ruined the record long before any symptoms set in. They failed to find a decrease in the cardiac output in six experiments in which there were only transient symptoms of faintness, lightheadedness or dizziness. On the contrary, the symptoms were often experienced during a period in which the cardiac output was definitely greater than that determined when they were absent. They state that a slight diminution of blood pressure was usually, but not always, observed concomitant with these symptoms; but in no case was the remaining pressure insufficient to raise blood to the top of the head.

We have used the roentgenkymographic method to calculate the standing cardiac outputs of eight adult males between the ages of twenty-one and forty. Three of these (H. S. M.; W. J. T., Jr.; W. D. D., Jr.) are non-fainters who can stand passively for at least twenty minutes with no manifestations of cardiovas-

[1] Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.

cular embarrassment. One, H. L. B., perspires freely, becomes pale and light-headed, but does not faint. The remaining four subjects have consistently shown signs of oncoming syncope within this period. The latter group includes one of the quarterbacks on the football team (T. G.), a former varsity football man (L. A. T.), and a former member of the boxing team (P. F. C.). Two of the subjects—H. S. M., a non-fainter, and L. A. T., a fainter—had served in the previously mentioned study in which the cardiac output alterations with posture had been determined by the acetylene method (1).

Roentgenkymograms were taken in mid-respiration with the subjects standing, using a target-film distance of 30 inches, exposure factors of 100 ma and 100 kv, and an exposure time of 1.5 seconds. The first kymogram was taken within three or four minutes after the subject was placed in position and was followed, in most instances, by a second exposure at the end of twenty minutes of quiet standing. When syncope seemed imminent before this time, an attempt was made to take the kymogram just before complete collapse. In one experiment (table 1, expt. 12), four films were taken at five minute intervals during the standing period. The diastolic and systolic volumes and the stroke output were calculated according to method B of Keys and collaborators.

Since our chief interest was in comparing the cardiac outputs at the beginning and at the end of the standing period, particular care was exercised in treating each pair of kymograms in the identical manner. Our use of the short focal distance (30 in.) introduced errors of distortion and magnification which cannot be adequately corrected. These were minimized, however, by use of the formula:

$$\left(\frac{D - X}{D}\right)^2 \cdot \text{Area (measured)} = \text{Area (corrected)},$$

D being the target-film distance (30 in.) and X, the heart-film distance. The latter value was taken as $\frac{1}{3}$ of the anterior-posterior chest diameter as measured with a pelvimeter, plus the distance from the outside of the kymograph panel to the film. In spite of the approximations inherent in our method, the values for cardiac output obtained agree rather closely with those previously determined by the acetylene method under similar conditions on the two subjects mentioned above. It should be emphasized, however, that the values given below are to be considered as having relative rather than absolute accuracy.

The results of our experiments are given in table 1. The relatively high values for stroke outputs are due to the fact that the kymograms were not taken under basal conditions and the majority of the subjects were not trained for the procedure. The lowest initial values in the series are those of the two veteran subjects, H. S. M. and L. A. T. The three non-fainters show only small changes in heart and stroke volume and in cardiac output. These may be considered to be within the limits of error of the method. The alterations in cardiac output vary with those in pulse rate. In two experiments the increase in the latter function results in a rise in the cardiac output; in the remaining three experiments the percentage decrease in cardiac output is greater than that in stroke output due to the diminished pulse rate at the end of the standing period. Our findings are

thus in general agreement with those of Starr and Rawson (4), who reported that the values for cardiac output in non-fainting subjects reach a plateau after a minute of standing, a level which is maintained with insignificant variations for at least ten minutes.

TABLE 1

SUBJECT	SURFACE AREA	EXPT. NO.	INTERVAL BETWEEN FILMS	HEART VOL. Diast.	HEART VOL. Syst.	Δv	STROKE OUTPUT ($1.44\Delta v$)	PULSE RATE	CARDIAC OUTPUT	P. C. CHANGE Stroke output	P. C. CHANGE Cardiac output	REMARKS
Non-fainters												
	m^2		*min.*	*cc.*	*cc.*		*cc.*	*min.*	*l./min.*			
H. S. M.	1.87	2-1		440.5	415.7	24.8	35.7	90	3.21			No circulatory em-
		2-2	20	454.0	431.5	22.5	32.4	86	2.79	−9.3	−13.0	barrassment
		11-1		436.7	406.0	30.7	44.2	88	3.89			No circulatory em-
		11-2	20	436.5	405.5	31.0	44.7	95	4.24	+1.0	+8.2	barrassment
W. J. T., Jr.	1.93	4-1		459.0	429.0	30.0	43.2	104	4.49			No circulatory em-
		4-2	20	462.2	431.6	30.6	44.1	100	4.41	+2.1	−1.8	barrassment
		9-1		389.0	356.0	33.0	47.5	104	4.94			No circulatory em-
		9-2	20	385.5	353.4	32.1	46.2	96	4.43	−2.7	−10.3	barrassment
W. D. D., Jr.	1.89	13-1		476.0	424.0	52.0	74.9	86	6.44			No circulatory em-
		13-2	20	462.5	414.3	48.2	69.4	96	6.66	−7.3	+3.2	barrassment
Intermediate												
H. L. B.	1.95	10-1		564.0	504.5	59.5	85.7	85	7.28			Dizzy, light-
		10-2	20	576.0	531.0	45.0	64.8	92	5.96	−24.4	−18.1	headed, *etc.* No fainting
Fainters												
T. G.	2.10	5-1		435.0	385.0	50.0	72.0	103	7.42			On verge of syn-
		5-2	18	417.0	379.3	37.7	54.2	104	5.64	−24.7	−23.9	cope
J. A. G., Jr.	1.67	6-1		402.0	354.7	47.3	68.1	106	7.22			On verge of syn-
		6-2	20	354.7	323.8	30.9	44.5	125	5.56	−34.7	−23.0	cope
L.A.T.	1.93	3-1		523.5	481.5	42.0	60.5	61	3.69			
		3-2	19	530.7	502.5	28.2	40.6	69	2.80	−33.0	−24.1	Fainted later
		8-1		497.4	461.3	36.1	52.0	68	3.54			Sweating, light-
		8-2	27	490.0	456.0	34.0	48.9	68	3.33	−6.0	−6.0	headed, dizzy.
		12-1		523.5	481.0	42.5	61.2	75	4.59			Considerable
		12-2	5	495.6	468.0	27.6	39.7	66	2.62	−35.1	−43.0	movement of arms
		12-3	10	523.5	490.0	33.5	48.2	75	3.62	−21.2	−21.1	On verge of syn-
		12-4	15	523.5	490.0	33.5	48.2	66	3.18	−21.2	−30.8	cope
P. F. C.	1.41	1-1		486.4	449.0	37.4	53.9	86	4.64			
		1-2	16	483.0	428.3	54.7	78.8	86	6.78	+46.2	+46.2	Fainted (see text)
		7-1		457.5	426.1	31.4	45.2	89	4.02			Considerable
		7-2	20	462.0	420.7	41.3	59.5	95	5.65	+31.6	+40.5	twitching, *etc.*

The findings for H. L. B., who was classified as an intermediate, can be correlated rather closely with his subjective manifestations. His stroke output decreased more than that of the non-fainters but less than that of the fainters. This drop in stroke output is minimized somewhat by the concomitant rise in the

pulse rate. With one exception to be discussed below, all of the fainting subjects show significant and marked decreases in their stroke and cardiac outputs. The changes in experiment 6 are of especial interest. After ten minutes of quiet standing, the subject was sweating profusely and was slightly dizzy. Signs and symptoms of syncope became increasingly manifest and the subject was urged to make every effort to remain in position for the twenty-minute test period. He was on the verge of collapse when the second kymogram was taken. Analysis of the kymograms indicates a decrease in diastolic and systolic volume of 11.7 and 7.1 per cent respectively and a marked drop in stroke volume. These changes are unquestionably correlated with the marked tachycardia evident at the end of the standing period. The increase in the pulse rate, accompanied by a diminished venous return, does not allow sufficient time for the adequate filling of the auricle. The ventricular volume becomes smaller and the stroke output is further decreased to such an extent that, in spite of the increased rate, the cardiac output remains low.

Experiment 12 on L. A. T. indicates that the stroke and cardiac outputs in fainters decrease considerably within a few minutes after the standing position is assumed, before any subjective signs or symptoms are evident. Thus the subject in this experiment reported feeling lightheaded and dizzy only after the third film was taken—about thirteen minutes after he assumed the standing position, and syncope was not imminent until five minutes later. Similar observations were made on two fainters examined under the fluoroscope. The changes in heart size were of sufficient magnitude to enable the observer to predict whether or not the subject would faint several minutes later.

The secondary rise in the stroke and cardiac outputs in experiment 12, as seen in the last two observations, is probably due to the restlessness evident when the standing period is prolonged beyond ten minutes, particularly in fainters who exhibit considerable swaying and involuntary movement of the arms after standing for seven to ten minutes. The importance of these muscular movements in increasing the stroke output even to the extent of preventing syncope is illustrated by experiment 8 on this same subject. This individual is our most consistent fainter, who, in experiments extending over four years, has seldom been able to maintain the position of quiet standing for twenty minutes without acute embarrassment. On this occasion he was perspiring freely and complained of being light-headed and dizzy at the end of eighteen minutes. He became restless, swayed and moved his arms considerably. Soon after, he reported feeling much better; and the second film, taken at the end of twenty-five minutes, showed a much smaller change in cardiac output than in other experiments.

Even more striking in this connection are the experiments on P. F. C. In the first experiment (expt. 1), the initial film was taken after three minutes of standing. Ten minutes later he was dyspneic, pale and showed fine fibrillary twitchings of the neck and arm muscles. He was sweating profusely and complained of a dry mouth. Two minutes after this time, the twitchings were much worse and he reported feeling weak and almost out. Five seconds later, before the second film could be taken, he fainted and would have fallen to the floor had he

not been securely strapped to the frame of the apparatus. He was twitching violently and exhibited a series of tonic and clonic convulsions. The period of unconsciousness lasted approximately a second, following which the muscular contractions subsided. The second film was taken at this time. The cardiac output calculated from it was 46.2 per cent higher than at the beginning of the standing. Similar results were obtained in a second experiment (expt. 7) on the same subject, who succeeded this time, however, in standing for twenty-three minutes without actually fainting.

These results strengthen the conviction based on previous experiments (6) that absence of adequate muscular contraction is the primary factor leading to a secondary failure of the circulation when standing is prolonged. During the period preceding collapse, decreased tissue temperatures, pallor, tachycardia and sweating, which are invariably present, attest the fact that the sympathetic centers are being stimulated rather than depressed. When an individual changes from the lying to the standing position there is a reduction in the distention of the aortic and sinus walls which evokes reflex compensatory responses such as a speeding of the pulse rate and a general vasoconstriction. The latter adjustment diminishes the volume flow and prevents a flooding of the capillary reservoirs in the subcardiac regions. Such a compensatory mechanism is only partially effective, however, for once the blood succeeds in getting through to the veins, it has difficulty in returning to the heart. In this way a vicious cycle is created. The volume flow is reduced not only as a result of the difficulty of returning blood to the heart against gravity but also by the compensating mechanism itself. If muscle tonus is low, there will therefore be a greater tendency for stagnation to occur and for the venous flow to be decreased to such an extent as to fail to meet the demands of gas transport for the individual at that particular time. The twitchings and muscular movements (sometimes convulsions) which often precede syncope are thus an attempt at compensation for the lessened venous return.

The usual classification of postural syncope as "vasovagal" is unfortunate, for cardiac inhibition is seldom seen until just before or during the syncope. Since the collapse occurs because of a diminished venous return it is obvious that changes in pulse rate would be contributory rather than causative factors. Marked changes in either direction would result in a decreased cardiac output. Thus the occurrence of syncope in experiments 3 and 12 on subject L. A. T. may be due to the presence of strong vagal tone preventing the usual compensatory increase in pulse rate. On the other hand, experiment 6 indicates that syncope may occur in the presence of tachycardia and suggests that the increase in the pulse rate may be great enough to shorten the time for diastolic filling appreciably so that, in the presence of a diminished venous return, the stroke output is markedly decreased. The cardiac output may thus fall because of the increased pulse rate.

As we have previously indicated, three of our fainting subjects were athletes in good physical condition. One of them, T. G., was playing football during the period he was serving as subject for these experiments. Syncope occurred in

these individuals at relatively high levels of cardiac output. Starr and Rawson (4) concluded that "there is a limit below which the cardiac output of a standing subject cannot go with safety, and that this limit is located near the middle of the normal range found in recumbent subjects, whose circulations are governed by different and less rigid requirements." Since the resting cardiac output of the athlete is usually higher than that of the non-athlete (5) it seems likely that the standing requirements of the former are higher than those of the latter. Athletes might thus be expected to show signs and symptoms of circulatory embarrassment at levels of cardiac output actually higher than those present in non-fainters under similar conditions. Under conditions in which the pumping and massaging actions of muscular contraction were at a minimum, athletes would be more severely handicapped than those individuals whose standing requirements are less and whose margin of safety in the standing position is therefore greater.

SUMMARY

Individuals who can stand quietly for at least twenty minutes show insignificant changes in the stroke and cardiac outputs during the standing period as determined by the roentgenkymographic method. Fainters show a marked decrease in these functions under the same conditions. If no marked movement occurs, the stroke output just before syncope, is 25 to 35 per cent less than at the beginning of the standing period, while the cardiac output has diminished 21 to 43 per cent. The development of syncope in quiet standing is primarily due to the absence of adequate muscular contraction which results in a diminished venous return. The vasomotor failure is secondary.

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DISAPPEARANCE CURVES OF THE DYE T-1824 AFTER ITS INJECTION INTO THE BLOOD STREAM[1]

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Literature on the determination of blood volume by the dye method reveals a conspicuous lack of agreement as to the interpretation of the dye curve (dye concentration vs. time after injection), and its utilization for calculating plasma volume.

The initial part of the time concentration curve is the resultant of both mixing of the dye with the blood and dye loss during this period. Direct quantitative determination of dye loss cannot be made during the period required for uniform distribution in the circulation. If the dye loss during this period is appreciable, it is evident that this must be allowed for in the calculation of plasma volume. As Erlanger (1921) pointed out, any determination of plasma volume by the dilution method is dependent upon knowledge of the exact rate at which the substance leaves the circulation.

The difficulty of deciding which particular straight line should be drawn through the time-concentration curve to represent average rate of dye disappearance during the distribution period led us to believe that further information on the dye curve was needed. The object of our investigation, therefore, was to obtain a more detailed knowledge of the time-concentration curve at every period, beginning immediately after injection and continuing for many hours thereafter.

PROCEDURE AND RESULTS. Some fifty experiments were performed on normal dogs, using the blue dye T-1824, as described by Gregersen and Stewart (1939). The dye was injected into a jugular vein, and a blood sample was drawn from the contralateral vein, sometimes only 30 seconds after injection. During the early stages samples were drawn frequently, often at half-minute intervals, but at longer intervals during the later stages. Dye concentrations of the samples were determined by spectrophotometric measurements of the serum.

Figure 1A gives examples of typical curves from experiments lasting about two hours, obtained by plotting the dye density of each successive sample as ordinate and the time after injection as abscissa. The form of the curves is in general similar to concentration curves for other of the more slowly disappearing dyes reported by previous observers (Erlanger, 1921; Smith, 1925; Robinow and Hamilton, 1940; and others). After the characteristic initial rise, the concentrations of successive samples decrease at a continually diminishing rate. Figure 1B shows that the same type of curve is maintained even when the observations

[1] Preliminary report: Federation Proceedings, Vol. 1, March 1942.

are continued for as long as 26 hours, the dye density continually decreasing during this period, but always at a progressively diminishing rate.

Discussion. Plasma volume is customarily calculated either from the dye density of a single blood sample or from the relation of successive samples. When the investigator relies on a single sample, the decision as to the moment of sampling is of great importance. The blood must be drawn before significant dye loss has occurred, yet it is clear from the curves that the concentration changes most rapidly during the first ten minutes after injection, so that this period offers a maximum opportunity for error.

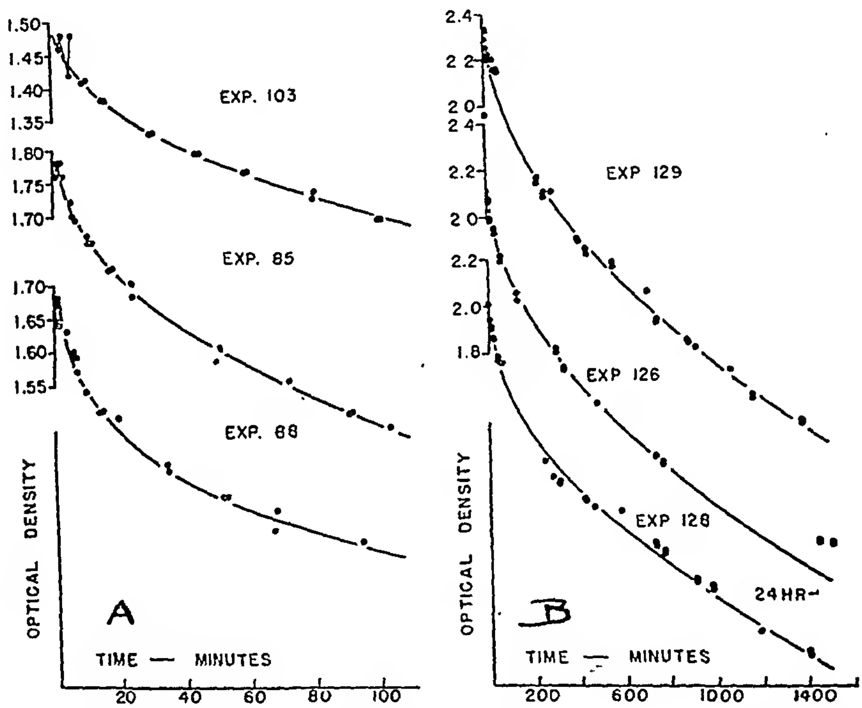

Fig. 1. Concentration curves of T-1824. Ordinates—optical density; abscissae—time, linear scale.

A, experiments lasting 100 minutes; B, experiments lasting 24 hours.

An alternative method, first suggested by Erlanger (1921), has been adopted by many observers (Sunderman and Austin, 1936; Gibson and Evans, 1937; Kennedy and Millikan, 1938; Gregersen and Stewart, 1939; Gregersen, 1941) in an endeavor to allow for dye disappearance during the mixing period. If, after mixing is complete, a curve can be established as representing dye loss from the blood stream, then backward extrapolation of this curve to the time of injection may be held to give the theoretical dye density at this instant. This is warranted only if the extrapolated line constitutes a true expression of the dye disappearance from the moment of injection.

It appears reasonable to assume that the rate of loss of dye injected in moder-

ate amounts would be proportional to the concentration, as is characteristic of many biological processes. This leads to the equation

$$c = c_o \cdot e^{-t/T} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where c_o is the concentration at the time of injection, c is the concentration at time t after injection, and T is a time constant. However, points obtained by plotting t and log c for data such as in figures 1 and 3 do not fall on a straight line, and equation (1) does not hold over any extended period, although a sufficiently short segment of these curves will always appear to be a straight line, particularly where the space allotted to the variables on the co-ordinates is dis-

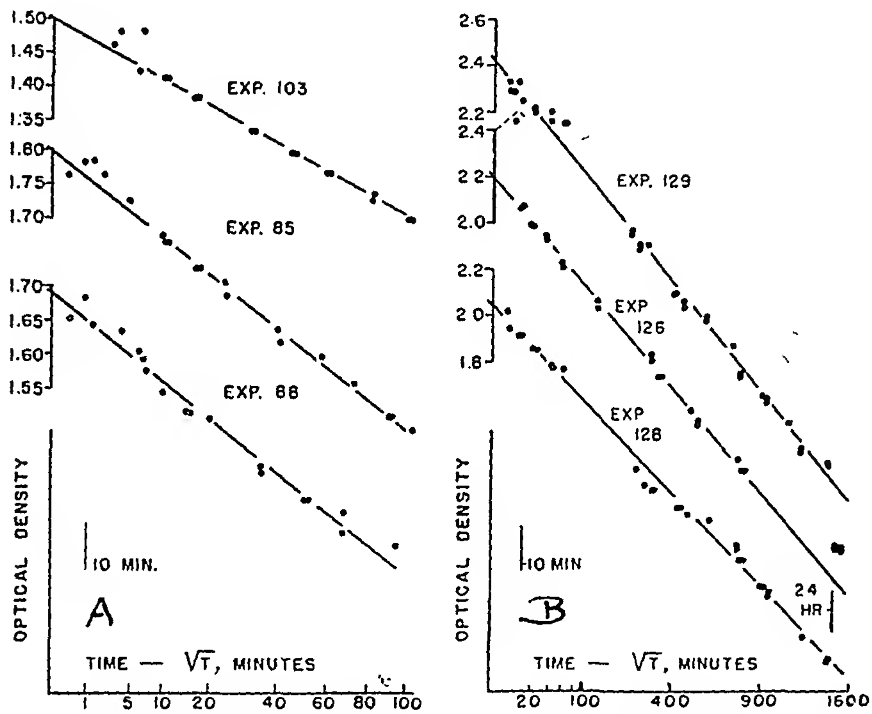

Fig. 2. Concentration curves of T-1824. Ordinates—optical density; abscissae—time, square root scale.

A, experiments lasting 100 minutes; B experiments lasting 24 hours.

proportionate. Dye concentration curves as often plotted are flattened as a result of emphasis on the time coordinate at the expense of the concentration coordinate. As a consequence, previous investigators have interpreted the selected portion of the curve as approximating a straight line given by

$$c = c_o(1 - t/T) \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where the symbols are as in (1). Figure 3 shows examples of this approximation. Its use for the determination of the initial concentration will be called the "linear extrapolation method," and will be designated by L.E.M. in subsequent discussion. The use of the L.E.M. for the calculation of plasma volume has already been criticized by Robinow and Hamilton (1940).

It may be seen from figure 3, curves I (Kennedy and Millikan, 1938), II (Gibson and Evans, 1937) and IV, that, if early points are included in the observations, they do not lie on the superimposed straight line. While it is evident that the mixing portion of a dye concentration curve must necessarily deviate from the true representation of dye loss during the distribution period, deviations from extrapolations of linear functions do not constitute an adequate criterion of mixing. Early points of a linear time-concentration curve always fail to fall on an imposed straight line, whether the period of observation is 12 minutes or 24 hours. This may not be immediately apparent from inspection of individual curves covering a limited time range, but will show up conclusively if the period of observation included in the curve is extended. This is illustrated also by figure 3; curves I and II were redrawn from the literature without modification, while curve IV was taken from one of our own experiments. The three straight

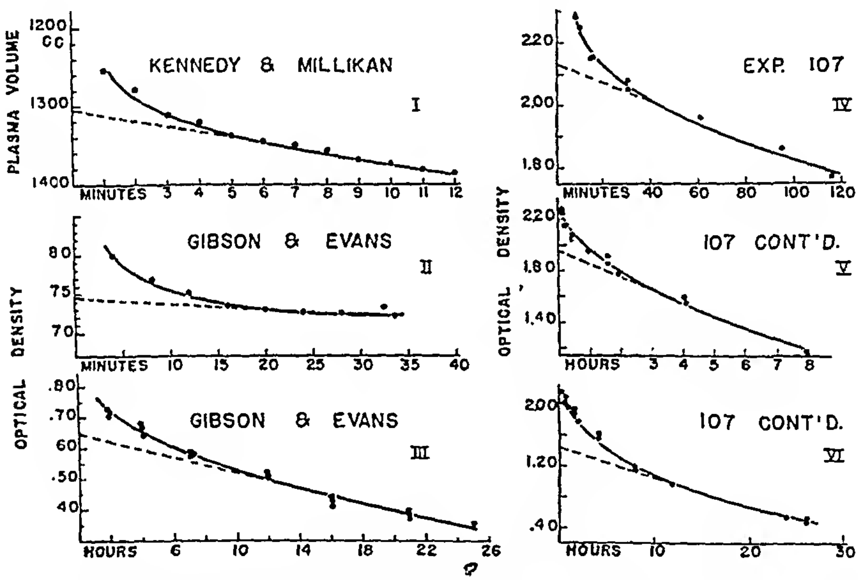

Fig. 3. Time-concentration curves plotted for varying periods after dye injection.

lines in curve III (Gibson and Evans, 1937), and in curves V and VI have been drawn in by us to show the deviation of points at the beginning of the curves. Such deviations, occurring several hours after injection of the dye, obviously cannot be attributed to incomplete mixing.

Since any limited portion of such a curve will serve equally well as approximating a straight line, the portion utilized has varied with the views of the investigator. In this approximation of dye loss as a linear function of time, the choice of the particular line of best fit must be revised whenever the time range is extended. This is illustrated in figure 3 by curves IV, V and VI from a single experiment, in which the interval after injection has been extended for each successive graph; the resulting extrapolated lines cross the ordinate axis at successively lower levels.

Since dye loss cannot be represented by a single linear function, the plasma volume calculated by the L.E.M. will vary according to the portion of the curve

from which the extrapolation is made. The earlier the period chosen, the steeper will be the imposed straight line and the lower the calculated plasma volume. Values of 41 cc., 47 cc. and 61 cc. respectively per kilogram were calculated from curves IV, V and VI, all from a single experiment. Even when far smaller differences in the periods of observation are employed, plasma volumes calculated by the L.E.M. may vary significantly.

By what means, then, can a knowledge of the dye dilution in plasma be utilized for estimation of plasma volume? Since the relatively slowly-disappearing dye, T-1824, passes from the circulation in recognizable amounts during the mixing period, a method which accounts for such dye loss is desirable. It should be remembered, however, that even using an adequate expression for dye loss after uniform dye distribution, there is at present no proof of the assumption that dye loss will be accurately represented by this same expression during the mixing period. Nevertheless, since there is no direct experimental method available at present for testing the relation during this period, we are dependent upon extrapolation as the best present method of calculating the dye concentration at the moment of injection.

The solution, in the opinion of the authors, lies in determining a relationship between dye disappearance and time which will adequately express average dye loss over an extended period, so that a single straight line may be obtained which can be extrapolated to the time of injection from any period.

The clue to such a relationship was found in curves such as IV, V and VI of figure 3. When the data for various intervals after injection were plotted on appropriate scales it was seen that the curves were practically identical in shape. This observation, and the ratios between the co-ordinate scales of these graphs, indicated that the dye loss was proportional to the square root of the time after injection. The dye concentration c, at time t, is then given by

$$c = c_0 \left(1 - \sqrt{t/T}\right) \qquad (3)$$

where c_0 is the initial concentration, and T is the time constant of the process. This equation was tested for its utility as a purely empirical device, and in the following discussion it will be called the "root extrapolation method," and designated by R.E.M.

The dye densities from 100 experiments[2] were accordingly replotted on the square root of time scale. The six experiments plotted linearly in figure 1 are shown in figure 2 plotted by the square root method. It may be seen that a single straight line suffices to represent average dye loss after an initial period of from three to ten minutes, regardless of whether the observation is continued for 100 minutes or for periods up to 24 hours. It is to be assumed that this single function will continue to describe dye disappearance for extended periods only where the physiological condition of the animal remains reasonably constant. It was found that during the longer experimental periods, however, certain procedures of convenience did not alter the straight line relationship. The dogs

[2] These include experiments performed by the authors, and others made available through the courtesy of Professor Gregersen.

were removed from the animal boards to their cages during prolonged intervals between sampling, they were permitted to move freely in their cages, to sleep, to drink water *ad libitum*, but undue muscular exercise and excitement were avoided.

Figure 4 shows experiment 107 plotted linearly and by the square root method. On the linear plot the three straight lines shown in curves IV, V and VI of figure 3 have been indicated. These serve to emphasize the increasing difficulty in the choice of a straight line of best fit when the period of observation is extended. The square root plot, on the other hand, establishes the choice of a line more conclusively with extension of the period of observation. In this experiment, where the distribution of points allows for some option in choice of a line, the extreme limits have been indicated in figure 4b. The plasma volumes calculated for these two extremes differ only by 2.13 per cent.

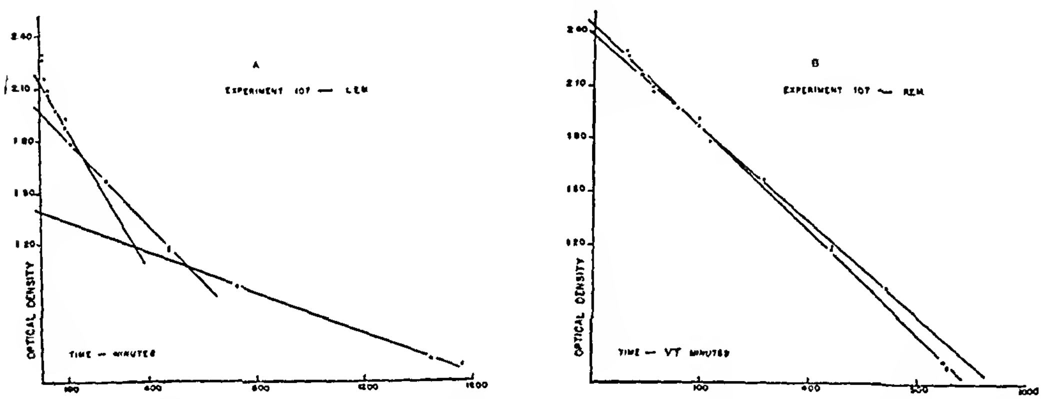

Fig. 4. A. Illustrates choice of line which may be drawn on a linear plot when the period of observation is extended.

B. The same data plotted as a function of the square root of time. Extremes of the choice of line are indicated.

Furthermore, when a square root curve, given by equation (3), is plotted on a linear time scale, the tangent is vertical at time $t = O$, so that, even without the complication of mixing, a linear plot of such a function is particularly difficult to extrapolate graphically to the initial concentration.

Plasma volume values for 100 experiments performed in these laboratories were calculated by both the L.E.M. and the R.E.M. The means and standard deviations for the two extrapolation methods were 52.6 $\pm$ 5.4 cc. per kilogram and 47.2 $\pm$ 5.3 cc. per kilogram respectively. There was great variability in the differences in individual values calculated by the two methods, hence values cannot be converted merely by application of a factor.

Standard deviations are approximately the same for the two series. Precision of this grade is attained with the L.E.M. only when the same or very similar time ranges are used for every experiment in the series. With the R.E.M. the precision will be the same whatever time interval within the first 24 hours is used for extrapolation.

The accuracy of the determination of the dye concentration at the moment of injection, and hence of the calculated plasma volume, necessarily depends upon the degree to which the average dye loss function employed represents dye loss during distribution.

If the square root function adequately describes average dye loss during the first three to ten minutes, then deviations of the points from the line within this period would indicate incomplete mixing. The reports of Graff, d'Esopo and Tillman (1931); Gibson, Keeley and Pijoan (1938) and Gilder, Muller and Phillips (1940) favor the view that a period of five to eight minutes is occupied by distribution of the dye. Observations by the authors confirm the view that mixing is complete within this period. If mixing is complete within two or three circulations, these deviations must depend on other factors (Robinow and Hamilton, 1940).

If the dye concentration were an exponential function of the time after injection as given by equation (1), we might have assumed that the dye loss was governed by a first order chemical reaction, or that the rate of dye loss from the blood stream was proportional to the concentration difference across a rather thin barrier. In the present case we turn to phenomena which involve the square root of the time, such as the "parabolic law" for the oxidation of metal surfaces, the "Schutz-Borissoff law" for enzyme kinetics, or the diffusion process described by the Fourier heat conduction equation. This latter process has interesting implications which cannot be discussed here.

Empirically, the equation (3) is an adequate representation of the data for periods from three to ten minutes up to 24 hours after injection. Within the range of the present data it may be seen that the disappearance curve is completely described by the constants c_0 and T.

It is obvious that equation (3) cannot be expected to describe the data for an indefinitely long time because c would become negative when $t = T$. Consequently the equation has additional terms which become important before $t = T$. The average value for T in the 100 experiments analyzed was found to be 36.4 hours (with the large standard deviation of ± 18.6 hours). These terms depend upon the mechanism and the geometry of the system and they have been calculated in several simple cases.

It is recognized that the concentration of dye in the blood is probably dependent upon several factors, such as: 1, the rate at which the dye, linked with albumin,[3] leaves the capillary bed and re-enters the circulation with the lymph; 2, the rate at which dye is excreted from the body, presumably through the activity of the liver; 3, the amount of dye held in readily staining tissues such as those of the lymph glands and kidneys, and whether such dye is available for later distribution. Mathematical functions used to describe the change in dye concentration over short or long periods may either represent the resultant of several processes or may express a limiting process.

We conclude that the time-concentration curve of the dye is best represented

[3] Rawson (in press) has shown that in presence of plasma proteins T-1824 is selectively linked with the serum albumin molecule.

as a function of the square root of time. Although this single function represents average dye disappearance during any period from ten minutes to twenty-four hours after injection, it cannot be stated dogmatically that this relation necessarily holds during the mixing period.

SUMMARY

1. The dye T-1824, when injected into the blood stream of the dog, disappears at a constantly diminishing rate.

2. Average dye loss cannot be adequately expressd by a single linear function for more than a very limited range of dye densities. As a consequence, deviation of the earlier points from such lines cannot be accepted as a criterion of incomplete distribution.

3. Values for plasma volume calculated from linear plots will vary with the period chosen for extrapolation, lower values characterizing determinations based upon periods shortly after dye injection.

4. Dye disappearance may be expressed adequately by a single straight line if dye densities are plotted as a function of the square root of time. This relationship maintains after the first five or ten minutes following injection and for any period up to twenty-four hours.

5. In contrast to the varying values obtained by extrapolation of different portions of the linear plot, the same value for plasma volume will be obtained during the first twenty-four hours whatever portion of the square root plot is used for extrapolation.

6. Plasma volumes calculated by the square root extrapolation method for any period between ten minutes and twenty-four hours average 10 per cent less than those calculated by the linear extrapolation method for periods between 30 and 120 minutes. The average percentage difference cannot be used as a factor for relating the two methods because the differences show considerable variability.

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DISTRIBUTION IN LEADS I, II AND III OF POTENTIALS APPLIED TO THE SURFACE OF THE HEART[1]

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A variety of experimental evidence has permitted two conclusions concerning the nature of the electrocardiogram: 1. Excitation at the surface of the right ventricle is recorded in the three conventional leads of the electrocardiogram as a monophasic-like complex, directed upward, while activation of the surface of the left ventricle produces a similar complex of opposite direction. The electrocardiogram is produced by the summation of these two components. 2. Lead I records the summation of the anterior levocardiogram and the posterior dextrocardiogram, while lead III records the summation of the anterior dextrocardiogram and the posterior levocardiogram. Lead II is the summation of leads I and III (1–8).

As a further test of the validity of these conclusions a study was made of the distribution in leads I, II and III of direct potentials applied to the surface of the ventricles. By this method it was possible to determine *a*, the direction of electrocardiographic displacement due to negativity at the surface of the right or left ventricle, and *b*, the leads in which potential differences applied at the various surfaces of the ventricles are preponderantly recorded.

Methods. Six dogs were employed, prepared as previously described (4). Pairs of circular tin electrodes 1 cm. in diameter were applied to the surface of the heart and were held in position by careful stitching through the epicardium or by the approximation of the pericardium. The electrodes were connected by fine insulated wires to a potentiometer across one or two dry cells. A commutator in the circuit made it possible to change the polarity at the electrodes. Potential differences with voltage low enough to avoid ventricular fibrillation were applied momentarily across these electrodes and the resultant beam deflections recorded in leads I, II and III of the electrocardiogram.

Results. *Effect of negativity and positivity at right and left ventricular surfaces.* When one electrode was placed on the right ventricle, and the other on the left ventricle, negativity of the plate at the surface of the right ventricle and positivity of the plate at the surface of the left ventricle caused an upward deflection in all three standard leads. Reversal of polarity so that the plate at the surface of the left ventricle was negative resulted in a deflection of equal magnitude but downward in all three leads.

Distribution of potentials in the several leads. *a.* When the electrodes were placed at the centers of the anterior surface of the right ventricle and the pos-

[1] Supported by a grant from the Fluid Research Funds, Yale University School of Medicine.

[2] Fellow of the Dazian Foundation.

terior surface of the left ventricle, and potentials applied regardless of polarity, a minimum deflection was found in lead I, which was occasionally unmeasurable. Lead III, however, showed a large excursion and lead II showed the summation of leads I and III (fig. 1, A, B).

b. When potentials were applied across electrodes placed at the centers of the anterior surface of the left ventricle and the posterior surface of the right ven-

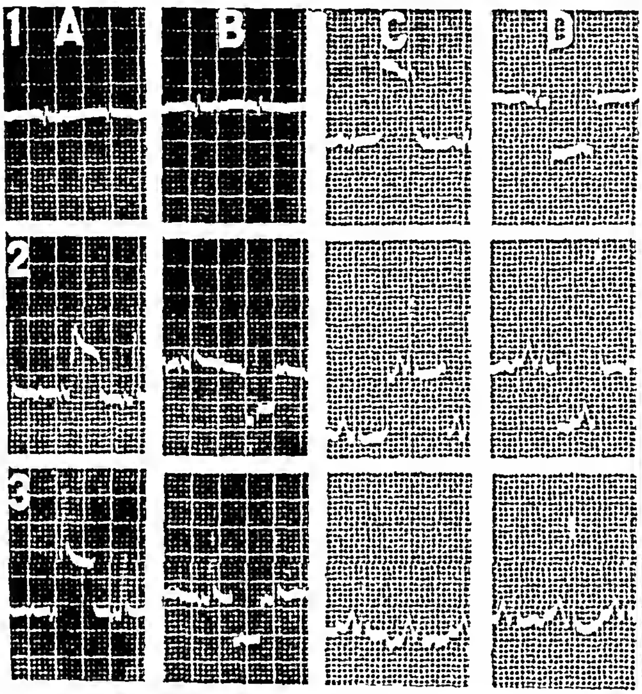

Fig. 1

Fig. 2

Fig. 1. February 11, 1942. Dog. 10.0 kgm. Dial anesthesia. Electrocardiograms from leads I, II and III showing make and break of applied potential. A. Plates on anterior right and posterior left ventricles. Negative plate *on right* ventricle. B. Plates as in A, but polarity reversed. C. Plates on posterior right and anterior left ventricles. Negative plate on right ventricle. D. Same as C, but polarity reversed.

Fig. 2. March 4, 1942. Dog. 7.5 kgm. Dial anesthesia. Antero-posterior x-ray of dog with plates in position. Triangular plates were placed on the anterior surface of the right ventricle and on the posterior surface of the left ventricle. Electrocardiograms of applied potentials showed preponderant effects in lead III (see fig. 1, A and B). The circular plates were placed on the posterior surface of the right ventricle and the anterior surface of the left ventricle. Electrocardiograms showed preponderant effects in lead I when potentials were applied, as seen in fig. 1, C and D.

tricle, regardless of polarity lead III showed minimal or no deflections, while in lead I large ones were recorded. Lead II again recorded the summation of leads I and III (fig. 1, C, D).

c. When the paired electrodes were placed (*a*) at the centers of the anterior surfaces of the right and left ventricles, (*b*) at the lateral margins of the right and left ventricles, or (*c*) at the centers of the posterior surfaces of the right

and left ventricles and potentials applied, leads I and III showed approximately equal deflections, in the same direction.

d. When four electrodes were placed at the centers of the anterior and posterior surfaces of the right and left ventricles, and the two anterior plates made negative, lead I showed a downward and lead III an upward deflection. When the posterior electrodes were made negative, the deflection was upward in lead I and downward in lead III. In both instances, lead II recorded the summation of leads I and III.

Surface projection of the axes recorded in leads I and III. In the above experiments (*a*, *b*, *d*) in which one pair of plates was placed on the centers of the anterior right and posterior left ventricles and the other pair on the centers of the posterior right and anterior left ventricles and potential differences applied across them, it can be considered that two electrical axes were established, one passing through the centers of the anterior left and posterior right ventricles, and the other through the centers of the anterior right and posterior left ventricles.

To permit visualization of the anatomical orientation of these axes, thin steel rods were inserted through the heart joining the centers of the paired plates. The projection of the axis joining the anterior right and posterior left ventricles on the horizontal plane of the body was roughly parallel to the longitudinal axis of the body or to a line joining the base of the left leg and left arm. The projection in the horizontal plane of the axis joining the centers of the posterior right and anterior left ventricles was roughly parallel to the transverse axis of the body.

Another way of visualizing the anatomical orientation of these axes is seen in figure 2. This figure shows an x-ray film of the thorax with the plates in position at the approximate centers of the anterior and posterior surfaces of the two ventricles. The lines joining the paired plates closely parallel the longitudinal and transverse axes of the body.

Discussion. It is generally accepted that in irritable tissue an active region is electronegative to an inactive area. In the heart it has been proposed that electronegativity associated with excitation in the right ventricle produces a monophasic action potential recorded in the three conventional leads of the electrocardiogram as an upward deflection, and that electrical activity in the left ventricle produces a similar complex directed downward. The experiments recorded here have shown that negativity when physically applied at the surface of the right ventricle does in fact evoke an upward deflection, while negativity similarly applied at the surface of the left ventricle causes a downward deflection.

The distribution of potentials applied to the surface of the heart follows closely that of potentials generated by the heart itself, i.e., lead I records preponderantly the potential changes between the anterior left and posterior right ventricles, while lead III records the potential changes between the anterior right and posterior left ventricles. This similarity in distribution appears to be accounted for by the virtual identity of the electrical axes with the anatomical axes joining the centers of the respective surfaces which are recorded in leads I and III.

It was seen that the projection in the horizontal plane of the axis joining the centers of the anterior right and posterior left ventricles was roughly parallel to the longitudinal axis of the body. Potential changes created at points along this axis would be expected to influence preponderantly lead III, and to have a minimal effect in lead I. Similarly, the fact that the axis of the anterior left and posterior right ventricles has a projection on the frontal plane at or near a right angle to the longitudinal axis of the body, explains why changes along this axis should have a minimal effect in lead III and a maximal influence in lead I.

It should be pointed out that such anatomical relationships vary in different specimens of the same species, in different species, and in each individual because of change in the position of the heart, etc. It should be expected, therefore, that the electrical representation of cardiac surfaces in leads I and III may be preponderant and not exclusive. This has been the case in these experiments as well as in those of different types which preceded them.

The observation that, when the anterior surfaces of both right and left ventricles are made electronegative simultaneously, an upward deflection is registered in lead I and a downward deflection in lead III, while opposite changes occur when the posterior surfaces are made negative, is a counterpart of earlier observations on the nature of leads I and III (5). It confirms conclusions drawn from them, namely, that the anterior surface of the right ventricle and the posterior surface of the left ventricle are represented preponderantly in lead III, while in lead I are recorded the electrical events at the surfaces of the anterior left ventricle and the posterior right ventricle.

SUMMARY

1. Potential differences applied across the surfaces of the right and left ventricles cause an upward deflection in the standard leads of the electrocardiogram when the negative electrode is on the right ventricle, and a downward deflection when the negative plate is on the left ventricle.

2. Potential differences applied across the centers of the anterior surface of the right ventricle and the posterior surface of the left ventricle affect preponderantly lead III of the electrocardiogram. The projection in the horizontal plane of the line joining these centers is roughly parallel to the longitudinal axis of the body.

3. Potential differences applied across the centers of the anterior surface of the left ventricle and the posterior surface of the right ventricle are recorded preponderantly in lead I. The projection in the horizontal plane of the line joining these centers is roughly parallel to the transverse axis of the body.

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NINHYDRIN, CRYSTALLINE PAPAIN AND FIBRIN CLOT

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Eagle and Harris (1) discovered that *papain* "acts directly on fibrinogen to form a fibrillar gel resembling fibrin." This has been confirmed (2) with a cyanide-activated *crystalline* papain enzyme, kindly supplied by Dr. A. K. Balls, U. S. Department of Agriculture. The same preparation was used in the present observations. A recent brief report (3) claims that fibrinogen can be clotted by the simple chemical agent *ninhydrin* (1,2,3-indantrione hydrate). In view of the importance of these data in the interpretation of the fundamental mechanisms of fibrin formation, they have been re-investigated with the aid of dark-field microscopy (cf. Stübel, 1914; Howell, 1914), in comparison with ordinary thrombic clots. Additional experiments by established methods (4) evaluate the possible rôle of ninhydrin in relation to blood-clotting mechanisms.

REAGENTS AND METHODS. Prothrombin and fibrinogen were prepared from citrated dog plasma and used according to the routine methods for experimental analysis of coagulation mechanisms previously described (4). The 0.9 per cent NaCl, used throughout as vehicle and diluent, was saturated with thymol. A 1:100 dilution of Parfentjev's (5) "rabbit clotting globulin" (Lederle Lab.) provided an excellent tryptase-free thrombin (T_G).

A lyophilized human fibrinogen (cf. 6) of high purity was supplied through the courtesy of Dr. E. J. Cohn (Harvard). It yielded clear, stable solutions completely free from thrombin and prothrombin and containing only a mere trace of serum-tryptase which caused "spontaneous" fibrinolysis of thrombic (T_G) clots in several days at room temperature. The fibrinogen was made up in phosphate buffer solution at pH = 7.7, somewhat diluted. Final fibrinogen concentration = 0.8 mgm. per cc. Crystalline papain: v. supra. Ninhydrin: a reliable German crystalline preparation was dissolved in distilled water to form a "stock" solution containing 20 mgm. per cc. With the larger quantities of ninhydrin the pH of the somewhat inadequately buffered fibrinogen was shifted almost to neutrality (phenol red indicator).

Data. Thrombin (T_G) and crystalline papain gave solid gels within a few seconds after adding to the fibrinogen solutions. The respective dark-field appearances are seen in figure 1, A and B. The typical fibrin needle-like meshwork appears to be identical in the two cases. The photomicrographs were made from a preparation of dog fibrinogen, nearly, but not quite, free from prothrombin. Exactly similar appearances were also obtained with the completely prothrombin-free human fibrinogen. Ninhydrin was likewise tested on both dog and human fibrinogen, the photomicrographs (fig. 1, C and D) being obtained with the cited dog material. Numerous strengths of ninhydrin were used, from contact of the fibrinogen (1 cc.) with undissolved crystals down to a (final) ninhydrin concentration of $\frac{1}{6}$ mgm. per cc. The mixtures invariably be-

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came turbid, in 1 to 2 minutes with the strongest ninhydrin and only after 1½ hours with the weakest strength employed. In all tubes a flocculent granular deposit formed later but at no time did any of the tests yield a gel sufficient to permit of inversion of the (11 mm.) tubes. However, the stronger concentrations developed a slimy supernatant that bore a crude resemblance to a "clot." Under the dark-field, two appearances were noted and are well illustrated in the photomicrographs.

The first (C) was a refractile granular deposit resembling that seen when fibrinogen or other proteins are "denatured" by heating or various flocculating agents. Sometimes the granules were larger, ovoid and budding (very like yeasts) or rounded and wrinkled, of size and appearance suggestive of crenated red blood-cells.

The second appearance (D) was an interesting thread-like formation (like mucus), the units of which were typically very long, straight, and arranged in parallel bundles. Sometimes they showed spiculated branching, and not in-

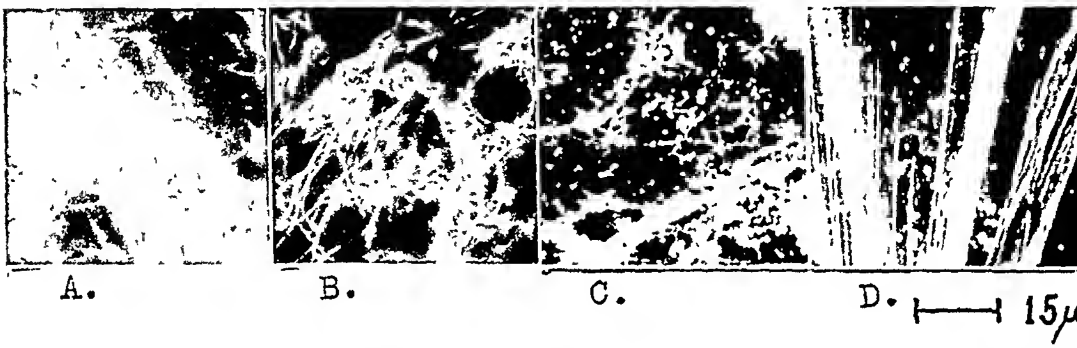

Fig. 1. Dark-field microscopy (oil-immersion lens) of fibrinogen (dog) mixed with: A, tryptase-free thrombin; B, crystalline papain; C; D, ninhydrin.

frequently there was evident a double outline and sometimes a series of varicose swellings.

It is obvious that these appearances differ widely from the typical fibrin meshwork and it is, therefore, concluded that ninhydrin is not in any real sense a thrombin-like substance capable of converting fibrinogen into true fibrin. The possibility remains that it might assist in thrombin formation in cruder systems containing thrombin precursors, in amounts greater than the traces identified in the cited dog fibrinogen. The following studies were made of the effects of ninhydrin on the isolated clotting mechanisms.

Ninhydrin and the thrombin-fibrinogen interaction (21.5°C.): Control: 1.0 cc. human fibrinogen + 0.25 cc. dist. water + 0.5 cc. thrombin (T_G) = clot in 28 sec. *Test:* 1.0 cc. human fibrinogen + 0.25 cc. ninhydrin + 0.5 cc. thrombin (T_G) = clot in 37 sec. The "stock" ninhydrin solution merely caused an unimportant delay in clotting which is attributable to a slight increase in acidity. A similar minor "second phase" effect can also account for the small differences in the earlier tests of A and B in table 1 (v. infra). Ninhydrin, therefore, is without significant effect on the thrombin-fibrinogen interaction.

Ninhydrin and the conversion of prothrombin to thrombin. Thrombic mixtures, each containing 4 cc. prothrombin and 0.9 per cent saline (as necessary) to make 5 cc., included the following, respective, *activators*: A. tissue thromboplastin (0.25 cc. dil. saline extract of frozen dog-brain) + 0.25 cc. N/10 $CaCl_2$; B. 0.25 cc. br. extr. + 0.25 cc. $CaCl_2$ + 0.25 cc. "stock" ninhydrin; C. 0.25 cc. $CaCl_2$ (alone); D. 0.25 cc. $CaCl_2$ + 0.25 cc. ninhydrin; E. 0.25 cc. ninhydrin (alone). Table 1 summarizes the activation tests, conducted at 21.5°C., pH = 7.7. Clotting-times are for 0.5 cc. samples of thrombic mixture, removed after the (incubation) periods cited and mixed with 1.0 cc. of a human fibrinogen solution, which control tests (*sans* prothrombin) proved to be completely free from prothrombin.

The ninhydrin-containing mixtures D and E developed a turbidity after 1½ hours and a subsequent fine granular deposit, but at no time was there any true clot formation. The extremely poor activation by calcium alone (C) shows that the prothrombin solution was all but free from thromboplastic factors. There is not the slightest evidence that ninhydrin can serve as a thromboplastic factor, alone or with calcium. The action of ninhydrin (B) during the first 20–30 min. activation phase in a complete thrombic system (prothrombin + Ca + added thromboplastin) consists of a negligible lessening of the apparent thrombic potency, especially in the early stages. This amounts only to a 1½ sec. difference at the "optimum" and in view of the above-noted minor second-phase effects, due to difficulty in controlling the acidifying tendency of the ninhydrin, may be dismissed as an inconsequential delay in the thrombin-fibrinogen interaction. It may be concluded that ninhydrin is without significant effect on the first phase of clotting also.

TABLE 1

Effect of ninhydrin on conversion of prothrombin to thrombin

Temp. = 21.5°C., pH = 7.7. Clotting-times (sec.) for 0.5 cc. thrombic mixture + 1.0 cc. prothrombin-free fibrinogen.

	INCUBATION PERIOD							
	5 min.	10 min.	20 min.	30 min	1 hr.	1½ hr.	2 hr.	13 hr.
1. Pro. + brain extr. + Ca.	23″	18″	15″	15″	18″	21″	22″	25″
2. Pro. + br. ext. + Ca + Nin.	34″	20″	16½″	16½″	26″	38″	56″	320″
3. Pro. + Ca.	trace 24 hr.	trace 24 hr.	trace 24 hr.	trace 24 hr.	trace 12 hr.	trace 12 hr.	± 12 hr.	+ 35 min.
4. Pro. + Ca + Nin.*	∞	∞	∞	∞	∞	∞	∞	∞
5. Pro. + Nin.*	∞	∞	∞	∞	∞	∞	∞	∞

* Flocculent turbidity in 1½ hr.

Ninhydrin and thrombinolysis; fibrinolysis. A comparison of A and B in the later stages (up to 13 hrs.) is interesting. Thrombin A was only very slightly unstable, due probably to a very small trace of thrombinolytic factor (? tryptase) in the crude brain thromboplastin. Thrombin B is definitely less stable. The clots of series A underwent fibrinolysis in 3 to 4 days, while those of series B

resisted lysis for 2 to 3 days longer. They were also more turbid. It may be concluded that ninhydrin, in common with innumerable other agents, alters the colloidal architecture of proteins and thus modifies the lytic phenomena, which are only incidental to clotting.

SUMMARY

Dark-field microscopy reveals the similar appearance of fibrin clots formed in prothrombin-free fibrinogen by 1, tryptase-free thrombin; 2, crystalline papain. The latter clots, unlike the former, undergo subsequent fibrinolysis. The dark-field appearances of the "pseudo-clots" formed by the action of ninhydrin on fibrinogen solutions, are quite different. An analysis of the behavior of ninhydrin in experimentally isolated clotting systems shows it to be without significant effect upon the fundamental coagulation mechanisms.

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THE INFLUENCE OF THYROID, DINITROPHENOL AND SWIMMING ON THE GLYCOGEN AND PHOSPHOCREATINE LEVEL OF THE RAT HEART IN RELATION TO CARDIAC HYPERTROPHY

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This investigation was undertaken in an attempt to gain insight into the chemical processes concerned in the development of cardiac hypertrophy. All observations were made on albino rats. It has been shown that this species develops an increased heart weight promptly upon feeding desiccated thyroid gland or thyroxin (1). This convenient method of altering cardiac size was used in the first series of experiments. In the second series of observations, attempts were made to influence the weight of the heart by the administration of dinitrophenol, and in the final series of experiments increased cardiac weight was induced by exercise.

Throughout the investigation all animals received a diet of Purina fox chow. At the termination of each experiment the rats were anesthetized by the intraperitoneal injection of pentobarbital sodium (50 mgm. per kgm. body weight).

I. *Hyperthyroid Cardiac Hypertrophy.* Previous investigators in the field of cardiac hypertrophy have not concerned themselves with the chemical changes in the *hyperthyroid* hypertrophied heart. The present experiments were designed to study the cardiac glycogen and phosphocreatine levels during the process of hyperthyroid hypertrophy.

1. *Glycogen concentration.* The glycogen concentration in the hypertrophied hyperthyroid heart has not been investigated, although Lawrence (2) and at least ten other workers have shown that in the acute thyrotoxic state, the cardiac glycogen levels are uniformly low.

In the present study, hyperthyroidism was induced by feeding dried thyroid glands (0.3 per cent organic I_2). The hearts, removed essentially according to the method of Evans (3), were analyzed for glycogen using a slight modification of Good's (4) procedure. The extent of cardiac hypertrophy was measured by comparing the heart weight/final body weight ratios (H.W./F.B.W.) of experimental and control animals. All analyses were made on 24-hour fasted animals.

The heart weight/body weight ratios and cardiac glycogen concentrations in all of the normal control rats studied were averaged. Seventy-four normal rats had a H.W./F.B.W. ranging from 2.6 to 4.5 $\times 10^{-3}$, averaging 3.2 $\times 10^{-3}$. In 36 normal rats the cardiac glycogen varied from 380 to 660 mgm. per cent, with an average value of 494 mgm. per cent. The standard deviation was found to be ± 68 mgm. per cent.

Feeding dried thyroid gland in daily doses of 1.0, 0.5 and 0.25 mgm. per gram of body weight, or feeding a diet containing 0.3 or 0.7 per cent thyroid to 102

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rats for periods varying from one week to six months resulted in progressive cardiac hypertrophy and progressive glycogen loss. The maximal H.W./F.B.W. was 8.1×10^{-3}, and the minimal glycogen value was 41 mgm. per cent. The degree of hypertrophy and glycogen loss was directly proportional to the size of the daily dose and the length of period during which it was administered. Apparently in the progressive development of hyperthyroid cardiac hypertrophy, no compensatory mechanism restored the glycogen in the heart to normal levels.

a. *Effect of age* (fig. 1): A series of observations was made to determine the effect of age on the response of the heart to thyroid feeding. Young (19 rats, 7 weeks of age at the start), adult (11 rats, 6 months of age), and senile (10 rats over 2 years of age) groups have been simultaneously studied. Each group received the same daily dose of thyroid, viz., 0.25

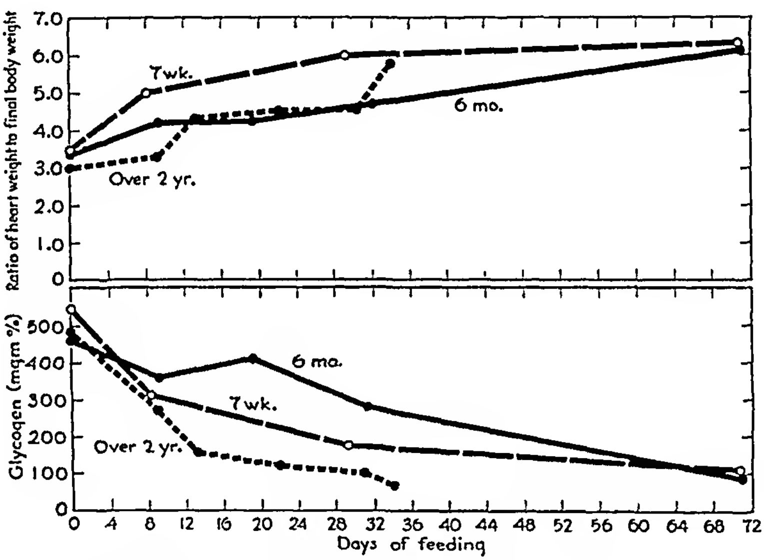

Fig. 1. Cardiac glycogen levels and H.W./F.B.W. $\times 10^3$ in thyroid-fed rats of varying ages.

Daily dose of thyroid: 0.25 mgm./gram of body weight.

mgm. per gram of body weight. Animals were killed at about one week, one month, and two months after beginning thyroid feedings. In the senile group none of the animals lived for two months, indicating some decrease in resistance to thyroid feeding in old rats. The three groups showed a comparable degree of cardiac hypertrophy. The results indicate that the development of the low cardiac glycogen concentration in the hyperthyroid hypertrophied rat heart is independent of the age of the animal. In all cases the cardiac glycogen was low in the presence of hyperthyroid hypertrophy.

b. *Effect of the technique used in removing the heart.* Electrocardiographic measurement of the heart rate in six hyperthyroid rats revealed a heart rate varying from 550 to 650 beats per minute, which is about twice the value found in normal rats of the same age. This acceleration of the energetic processes should cause an increased oxygen requirement of the heart. Chang (5) has shown that the cardiac glycogen in the normal rat is rapidly depleted by anoxia and adrenalin. Because of these factors it appeared possible that the low glycogen concentration observed in the hyperthyroid heart resulted from hypersensitivity to the anoxia and adrenalin incident to the removal of the heart which required three to five seconds. In an attempt to eliminate the effects of anesthesia and surgery, and there-

by anoxia and adrenalin liberation, the following experiments were performed: A device was made in which it was possible to instantaneously sacrifice and transect the untreated animal with a single stroke through the chest. The exposed heart was immediately transferred to hot potassium hydroxide solution. In three normal rats so treated the cardiac glycogen level averaged 465 mgm. per cent, which agrees satisfactorily with the value obtained above. The hearts of three hyperthyroid rats (fed 0.5 mgm. thyroid per gram of body weight daily for 17 days) when removed by this procedure, showed an average level of 110 mgm. per cent, which again is in the same range as the values obtained using the standard method.

In order to eliminate possible effects of adrenalin and to shorten the anoxial period during removal the following method of removing the heart was designed. Under ether anesthesia the rats were bilaterally adrenalectomized. Seven to ten hours later the rat was anesthetized with pentobarbital sodium, artificial respiration established, and a single midsternal incision made. After 15 to 20 minutes a mixture of ether and solid carbon dioxide was poured on the heart. Nine normal rat hearts removed in this way showed an average glycogen concentration of 497 mgm. per cent. Using this technique the cardiac glycogen values in the hyperthyroid animals were again low. Five rats, fed a 2 per cent thyroid diet for six days, showed an average glycogen concentration of 178 mgm. per cent. The mean H.W./F.B.W. was 4.5×10^{-3}. These two experiments support the view that thyroxin has a specific glycogenolytic action, and that throughout this series of experiments neither the anesthesia nor the temporary anoxia associated with removal of the hearts appreciably influenced the results.

2. *Phosphocreatine* (table 1). No studies were found on the phosphocreatine level in the hypertrophied hyperthyroid heart. As in the case of glycogen, investigators have been interested in the acute hyperthyroid state in which cardiac hypertrophy is absent. Schumann (6) studying rats found variations in normal levels of cardiac phosphocreatine of from 9.9 to 13.9 averaging 11.0 mgm. per cent P. When 4 mgm. of thyroxin were given for a period of four days he found the level dropped to 4.8 mgm. per cent. Chanutin (7) showed that a 10 per cent creatine diet fed for two days elevated the normal rat heart creatine concentration from the control value of 217 mgm. per cent to 282 mgm. per cent. The effect of creatine feeding on the level of phosphocreatine in hyperthyroid hearts has not been reported.

The following experiments were undertaken to determine, first, the phosphocreatine concentration in *hypertrophied* hyperthyroid rat hearts, and second, the effect of feeding creatine upon the level of phosphocreatine in such hearts. The analytical procedure followed was that of Eggletons (8), using an Evelyn photo-electric colorimeter. It was found necessary both to freeze the hearts immediately in an ether-solid CO_2 mixture and to carry out the extraction at very low temperatures. The results in ten normal rat hearts ranged from 10.0 mgm. per cent to 14.5 mgm. per cent of phosphocreatine phosphorus with a mean of 12.0 mgm. per cent (table 1).

The rats, individually caged, received identical amounts of food. Two groups of rats fed a diet containing 0.3 per cent thyroid for 18 and 20 days showed cardiac hypertrophy. In the first group, with a H.W./F.B.W. of 4.3×10^{-3}, the phosphocreatine concentration was 4.9 mgm. per cent (table 1). In the second group with a marked degree of hypertrophy, H.W./F.B.W., 6.2×10^{-3}, the phosphocreatine level was 5.9 mgm. per cent. It is clear that the low levels of cardiac phosphocreatine, as well as glycogen, found in acute hyperthyroidism persisted during and after the development of cardiac hypertrophy.

A third group of five rats were maintained on a diet containing 0.3 per cent thyroid and 6.0 per cent creatine for 18 days. The average phosphocreatine concentration was 3.9 mgm. per cent as compared to a control group, receiving thyroid but no creatine, with an average of 4.9 mgm. per cent. A fourth group received a 0.3 per cent thyroid diet for 20 days, a 10 per cent creatine diet being substituted on the last day. The phosphocreatine level was on the average 1 mgm. per cent above the average control value of 5.9 mgm. per cent. It was concluded that creatine feeding did not significantly alter the cardiac phosphocreatine level of the hyperthyroid hypertrophied rat heart.

II. *Attempts to Produce Cardiac Hypertrophy by Slowly Absorbed Dinitrophenol.* Taussig (9), in experiments on rats, administered 10 to 15 mgm. of dinitrophenol per kilogram body weight twice a day for periods varying from 2 to 17 days. He found the cardiac glycogen concentration to be normal. Fatal doses produced a reduction in cardiac glycogen which he suggested was probably due to anoxemia. Wesselow (11) administered 50 mgm. of dinitrophenol daily in the food for one month to 200 gram rats in an unsuccessful attempt to produce cardiac hypertrophy. To determine if parenterally administered dinitrophenol is also ineffective in producing hypertrophy, the following experiments were conducted.

Dinitrophenol was incorporated into the beeswax-mineral oil mixture which

TABLE 1

Phosphocreatine concentration in the normal and hyperthyroid hypertrophied rat heart

DIET	F.B.W.	$\frac{H.W.}{F.B.W.} \times 10^3$	PHOSPHOCREATINE (MGM. PER CENT P)	DIET	F.B.W.	$\frac{H.W.}{F.B.W.} \times 10^3$	PHOSPHOCREATINE (MGM. PER CENT P)
Control	260	3.8	12.0	0.3% thyroid, 20 days	260	5.5	6.2
0.3 per cent thyroid, 18 days	310	3.7	4.3		315	4.9	5.9
	290	4.8	5.2		220	6.6	6.4
	310	4.5	4.5		170	8.0	5.1
	310	4.0	5.1				
	310	4.3	5.2				
Average.............		4.3	4.9	Average.............		6.2	5.9
0.3 per cent thyroid, 6.0 per cent creatine, 18 days	320	3.7	2.6	0.3% thyroid 20 days + 10% creatine last day	250	5.1	6.2
	270	5.8	5.1		250	5.3	7.5
	285	4.8	4.7		230	5.4	6.9
	285	4.2	2.5				
	285	5.0	4.5				
Average.............		4.7	3.9	Average.............		5.3	6.9

Code and Varco (11) found to be satisfactory for delaying histamine absorption. Six rats were each given 275 mgm. of 2-4 dinitrophenol subcutaneously in divided doses over a period of 16 days. No significant cardiac hypertrophy resulted, the average H.W./F.B.W. being 3.7×10^{-3}. The animals showed a 15 per cent loss in weight. The cardiac glycogen levels were essentially normal, the mean being 425 mgm. per cent, with a range from 275 to 531 mgm. per cent.

III. *Glycogen Concentration in Hypertrophied Hearts of Swimming Rats* (*table 2*). Kirch (12) has demonstrated in the rat that swimming can result in cardiac hypertrophy but no chemical analyses of such heart muscle have been reported. Accordingly the following studies were made to determine if the concentration of glycogen was altered by the development of this type of cardiac hypertrophy.

Young male rats (60–80 grams) were selected for this experiment. These animals were divided into six groups, all receiving the same diet. The first two groups were unexercised and served as controls. The remaining groups (groups

3, 4, 5, and 6) were put in a water bath at 25°C. for an average of 3.3 hours per day. After periods of 50 and 60 days the animals were sacrificed under pentobarbital with or without a previous 24-hour fast.

The hearts of the exercised animals were consistently heavier than those of the control animals. The heart weight: body weight ratio was significantly increased in the swimmers and this was not due to a loss of body weight. In

TABLE 2

Glycogen concentrations in hypertrophied hearts of swimming rats

GROUP	F.B.W.	$\frac{H.W.}{F.B.W.} \times 10^3$	GLYCOGEN (mgm. per cent)	GROUP	F.B.W.	$\frac{H.W.}{F.B.W.} \times 10^3$	GLYCOGEN (mgm. per cent)
1. Controls (fasted 24 hrs.)	160	4.0	444	4. Swimmers (not fasted) 60 days. 3.3 hrs./day. Killed 1–2 hrs. after swim	190	4.7	434
	210	3.4	531		184	4.4	556
	160	3.7	470		170	5.1	652
	220	3.5	565		150	5.7	648
					160	5.5	690
Average		3.6	502	Average		5.3	596
2. Controls (not fasted)	160	3.8	413	5. Swimmers (not fasted) 60 days. 3.3 hrs./day. Killed 24 hrs. after swim	180	4.6	510
	175	3.7	391		185	4.4	432
	190	4.3	550		180	4.5	388
	165	3.6	635		150	5.5	467
	150	4.5	485		120	4.6	482
	135	4.2	605		190	4.2	513
	180	3.7	412				
Average		3.9	498	Average		4.6	465
3. Swimmers (fasted 24 hrs.) 50 days. 3.3 hours/day. Killed 24 hrs. after swim	150	5.5	1120	6. Swimmers (not fasted) 50 days. 3.2 hrs./day. Killed 24 hrs. after swim	180	4.3	859
	180	5.1	1300		180	5.0	695
	140	5.2	1345		200	4.7	662
	165	5.3	790		200	4.4	671
	160	5.4	819		175	4.8	731
	145	5.5	1305				
Average		5.3	1013	Average		4.6	723

contrast to the results obtained with hyperthyroid hypertrophy, the glycogen concentration in the hearts of the exercised animals was normal or increased. In the swimmers (group 3) which had been fasted and rested for 24 hours prior to killing, the cardiac glycogen concentration averaged twice as high as normal. In the rats (group 4) killed 1 to 2 hours after swimming without fasting, the cardiac glycogen was normal. Conflicting results were obtained in groups 5 and 6. In both of these groups the rats were not fasted and were killed 24 hours after swimming. Group 5 showed essentially normal glycogen values while group 6 showed elevated levels. No explanation can be offered at the present

time for this variance. It has been concluded that in young rats swimming can produce hypertrophied hearts which show normal or increased glycogen concentrations 1 to 24 hours after swimming.

COMMENT. The experiments in which cardiac hypertrophy occurred in response to thyroid feeding make an interesting contrast to those in which hypertrophy occurred in response to exercise. When thyroid was fed over a prolonged period, the rat developed marked cardiac hypertrophy. When the rat was exercised each day for about three hours a moderate degree of hypertrophy occurred. In the thyroid-fed rat the cardiac glycogen fell to a low level and remained low throughout the entire experiment. The exercised animals were killed after a 1 to 24-hour rest and the glycogen level was then equal to or above the control levels. In the thyroid-fed rats the stimulating effects of the ingested thyroid acted continuously throughout the entire period of observation while in the exercised rats the metabolic stimulant was applied for only about three hours of each day. The glycogen level in the hearts of the exercised animals may have been low at some time during the three-hour period of exercise, but it was not tested. Actually, the exercised animals which had been fasted and rested for 24 hours showed the highest glycogen levels which we have encountered in any rat heart. It is important to note that no exercise studies were made in the adult rat which may show a different response.

In the experiments in which thyroid was fed it seemed there might be a relation between low cardiac glycogen levels and the development of cardiac hypertrophy. When the thyroid was fed, low glycogen concentrations in the heart preceded and accompanied the development of hypertrophy. During prolonged thyroid feeding the cardiac glycogen concentration remained low. Thus, in these experiments the progressive development and maintenance of hyperthyroid cardiac hypertrophy was not accompanied by a compensatory restoration of the glycogen in the heart.

SUMMARY

1. Both glycogen and phosphocreatine were found to be present in low concentrations in the *hypertrophied* hyperthyroid rat heart.

2. Dinitrophenol administered subcutaneously for a period of two weeks failed to produce an increase in the heart weight of rats.

3. Young rats which had been swimming 3.3 hours daily for about 2 months showed moderate cardiac hypertrophy. When determined 1 to 24 hours after swimming the cardiac glycogen was normal or elevated. When the animals had been fasted and rested 24 hours after swimming the cardiac glycogen concentration averaged twice as high as normal.

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EFFECTS ON MAN OF SEVERE OXYGEN LACK

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Interest in the response of schizophrenic patients to oxygen lack has existed for several years. Experience with various shock therapies has brought forth the view that their effects may be related to the induction of cerebral anoxia. This appears to be the case in the insulin shock treatment of Sakel (1) and in the nitrogen breathing treatment introduced by Himwich and associates (2). The rationale of the anoxia therapy used in our investigation is being presented by Corwin and Horvath elsewhere (3) and requires only brief mention here.

Since the results in any shock treatment depend on the duration as well as the intensity of the stimulus, we have employed mixtures of nitrogen and oxygen low enough in oxygen to produce unconsciousness and yet capable of sustaining life for many minutes. The intensity and duration of the stimulus was varied to suit the individual case. The principal gas mixtures used contained 4.2, 5.2 or 6.0 per cent oxygen. The duration of exposure to these mixtures varied from 3 to 16 minutes, 6 to 21 minutes and 4 to 15 minutes, respectively. Unconsciousness frequently terminated those treatments that involved either 4.2 or 5.2 per cent oxygen. It was rarely observed during the time intervals indicated with 6.0 per cent oxygen. In some instances there was an induction period during which the patient breathed 14 per cent oxygen. Recovery was effected usually by abruptly shifting from the gas mixture to air while on occasions 14 per cent oxygen was supplied during the first stage of recovery. Male patients were used as described in table 1.

The observations made include: *a.* Measurement of respiratory volume and rate before, throughout and after the anoxic period. *b.* Sampling and analysis of expired air before, during and after the anoxic period. *c.* Sampling and analysis of arterial blood before, during and after the anoxic period. *d.* Hematological responses. *e.* Pulse and blood pressure. *f.* Electrocardiograph. *g.* Mental state of the patient. Observations *a* to *c* provide the principal factual data on which this report is based.

Results. The measurements of respiratory volume in those treatments involving 6.0, 5.2 and 4.2 per cent oxygen are shown in figures 1 to 3, respectively. In the preliminary period, hyperventilation was frequently observed. An unexcited person at rest will breathe from 5 to 8 liters per minute: the fact that some of these patients reached values as high as 15 liters per minute, the

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average being about 10, is evidence of excitement and/or physical activity. Some remained quiet and unexcited, others were quiet but tense while a few were disturbed and resistant. Every possible precaution was taken to keep the face mask in place throughout but some tests failed because this was impossible.

There are several notable features of the results. There was invariably an

TABLE 1

Description of patients

The diagnosis was dementia praecox. The first seven were catatonic, the others hebephrenic.

SUBJECT	AGE	HEIGHT	WEIGHT	LENGTH OF HOSPITALIZATION
	years	*inches*	*pounds*	*years*
B. H. E.	26	66	122	8.0
C. J. G.	25	66	122	3.5
S. J. A.	27	66	176	8.0
C. W. A.	34	70	156	8.4
D. C.	32	71	185	6.0
D. F. W.	24	70	129	6.7
H. C. N.	23	71	128	8.5
M. D. R.	28	65	131	4.5
P. F.	34	67	140	16.7
Q. B. J.	33	66	120	5.6
C. J. H.	31	72	160	10.8

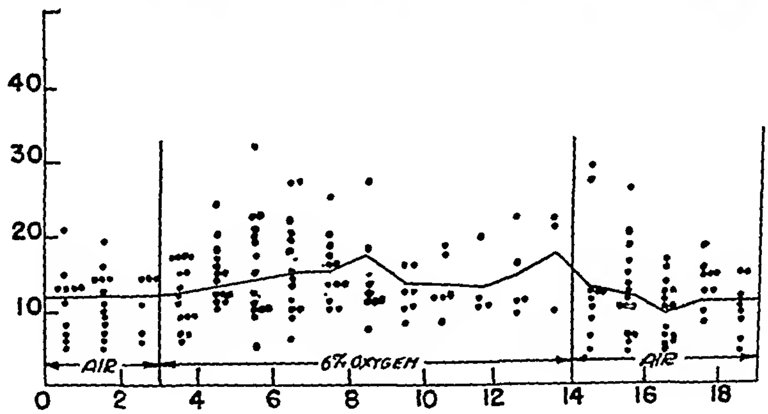

Fig. 1. Pulmonary ventilation in liters per minute before, during, and after breathing 6.0 per cent oxygen. The curve corresponds to the average response. Time is in minutes.

increase in pulmonary ventilation within two minutes after starting to breathe 4.2 per cent oxygen. The general trend throughout the first five minutes of anoxia was upwards, reaching, on the average, 30 liters per minute and in one case, during the sixth minute of anoxia, 65 liters per minute. In not a single instance did the respiratory volume during the anoxia produced by breathing 4.2, 5.2 or 6.0 per cent oxygen drop below the level observed in the preliminary control period.

A second striking feature is the wide range in the respiratory volumes. This is a well-known characteristic of the response of healthy men to anoxia. It accounts in part for the variability in their ceiling.

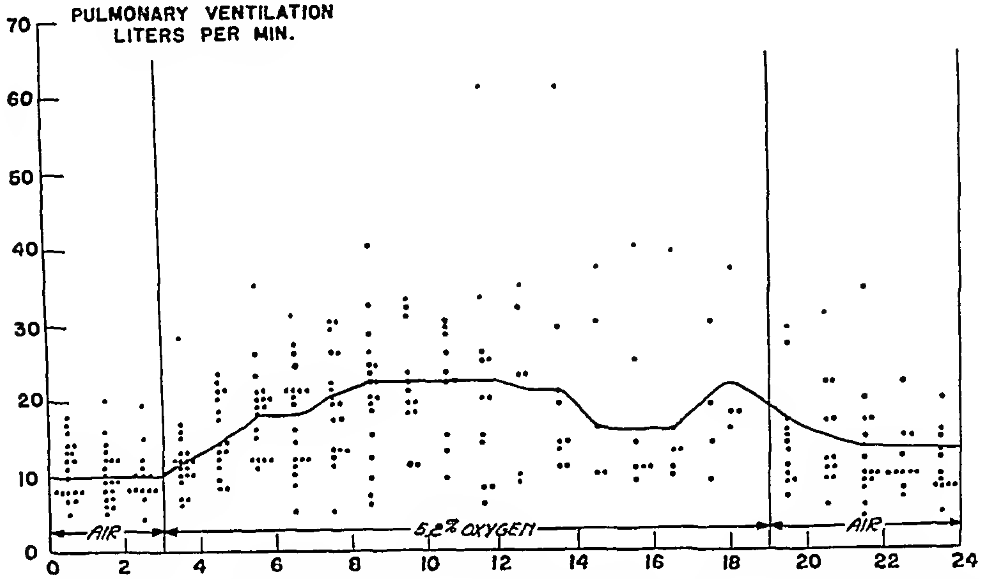

Fig. 2. Pulmonary ventilation before, during, and after breathing 5.2 per cent oxygen.

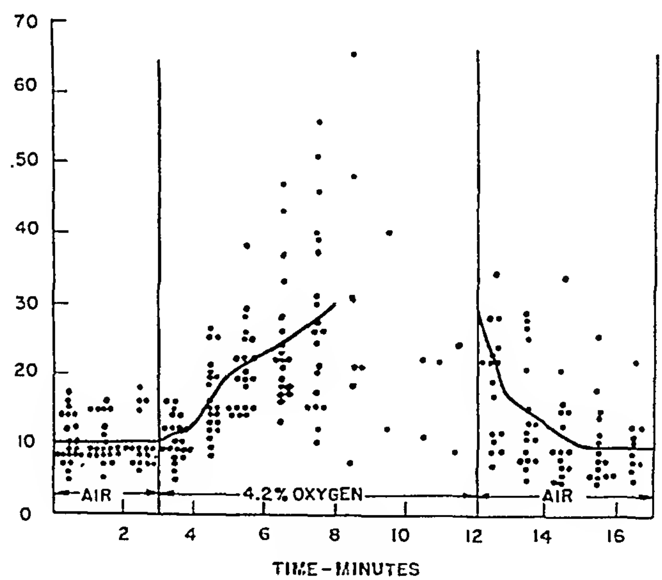

Fig. 3. Pulmonary ventilation before, during, and after breathing 4.2 per cent oxygen.

The third and most striking feature is the slow decline towards normal breathing in recovery. It is taught by physiologists that a period of overventilation is followed by a period of diminished ventilation or of apnea. No such decline was noted during the first five minutes of recovery in these patients. Following sudden access to air of normal composition, breathing usually continued above

the initial level for from 1 to 5 minutes and in no instance dropped to an alarmingly low level. This matter will be discussed below. In those cases where a shift was made from low oxygen to 14 per cent oxygen the respiratory response was the same as when the return was made to air.

Any considerable increase in respiratory volume during anoxia was accompanied by an increase in respiratory rate and depth. Schizophrenic patients characteristically have a rate above the range of most healthy individuals but in extreme anoxia the rate is increased even more—up to 50 times per minute in some instances. The mean values for three degrees of anoxia are shown in figure 4.

The composition of expired air indicates that a steady state was not attained in these experiments. The percentage of CO_2 in expired air continued to de-

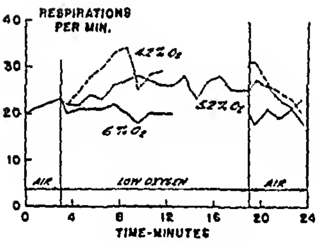

Fig. 4

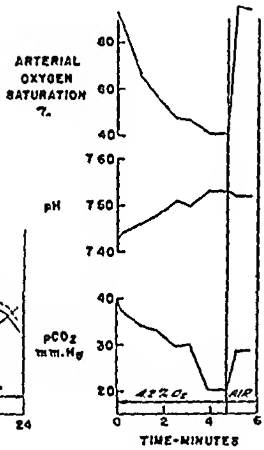

Fig. 5

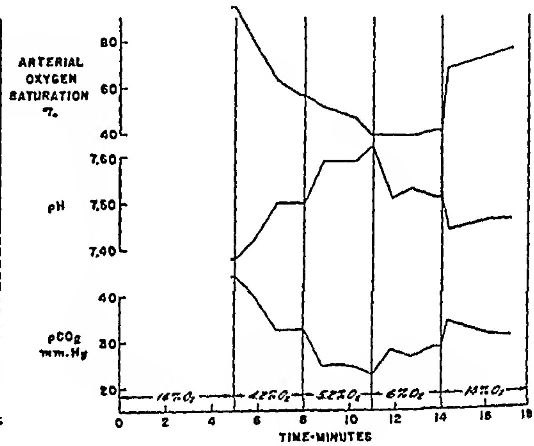

Fig. 6

Fig. 4. Respiratory rates before, during, and after periods of anoxia.

Fig. 5. Properties of arterial blood during and immediately after a five-minute exposure to 4.2 per cent oxygen.

Fig. 6. Properties of arterial blood while breathing atmospheres of various oxygen contents. The results indicate the interdependence of arterial oxygen, carbon dioxide, and degree of alkalosis.

cline. The decline in percentage of oxygen in expired air was abrupt at first and then more slow. A typical experiment is summarized in table 2.

Respiratory regulation at the onset of unconsciousness is revealed by the composition of expired air and respiratory rate and volume. These data, obtained in 20 tests on 7 subjects are collected in table 3. These data support the evidence given in figures 1 to 3 that the onset of unconsciousness in deep anoxia is not associated with respiratory arrest. Without exception the respiratory minute volume and the respiratory rate are high even though in some instances the gas expired contained less than 3.5 per cent oxygen.

Arterial blood was drawn during the height of the anoxia. Its analysis revealed oxygen saturations often as low as 50 per cent and sometimes below 40 per cent. Associated with the increased pulmonary ventilation there was ob-

TABLE 2

Time course of respiratory changes during inspiration of 4.2 per cent oxygen

Subject C. W. A., July 29, 1941

TIME	COMMENTS	COMPOSITION OF EXPIRED AIR		VENTILATION AT 760 MM. Hg 37°C.	RESPIRATORY RATE
		O_2	CO_2		
min.		*per cent*	*per cent*	*l./min.*	
0	Observations begun				
4	Breathing air	17.83	2.91	7.4	18
5	4.2% O_2 begun				
6		3.22	5.06	14.8	22
7		2.13	4.14	12.3	22
8		2.77	3.61	14.8	19
9	Unconscious	2.55	3.59	22.9	29

TABLE 3

*Gas samples obtained at the onset of unconsciousness**

DATE	SUBJECT	OXYGEN IN GAS INSPIRED	COMPOSITION OF GAS EXPIRED		PULMONARY VENTILATION AT 37°C., 760 MM. Hg.	RESPIRATORY RATE
			CO_2	O_2		
1941		*per cent*	*per cent*	*per cent*	*l./min.*	
July 23	B. H. E.	4.2	2.44	3.38	16.5	27
28		4.2	2.25	3.21	10.2	33
Sept. 5		4.2	2.07	4.79	19.6	21
10		5.2	1.67	3.80	25.4	35
15		6.0	1.36	4.51	11.2	40
July 22	C. J. G.	5.2	0.92	4.74	18.8	26
24		4.2	2.10	3.81	18.0	30
Sept. 9		4.2	1.68	4.22	21.7	38
11		4.2	2.13	3.45	18.2	36
July 22	S. J. A.	5.2	2.50	3.94	31.4	39
24		4.2	2.02	3.63	32.7	42
29		4.2	2.63	3.51	22.0	15
Sept. 16		4.2	2.59	4.38	47.5	37
July 17	C. W. A.	5.2	2.76	4.09	15.4	19
24		4.2	2.79	3.42	27.9	29
Sept. 9		4.2	2.95	3.07	23.3	19
July 23	D. F. W.	4.2	2.68	3.58	36.5	33
July 24	Q. B. J.	4.2	2.74	3.97	23.9	32
Sept. 9		4.2	2.50	3.99	26.8	31
Sept. 16	C. J. H.	4.2	2.95	3.35	21.0	19

* The gas samples were collected during a 30-second period. Eight were obtained just prior to unconsciousness and 12 extended into the period of unconsciousness.

served in the blood a decreased content of carbon dioxide and an increase in alkalinity, the pH often reaching 7.6. Some typical data are summarised in table 4, and the course of a typical run in which 4.2 per cent oxygen was breathed for 5 minutes is shown in figure 5.

In one type of test the patient was supplied with 14 per cent oxygen and then progressively with 4.2, 5.2 and 6.0 per cent oxygen each for 3 minutes. A needle was left in the artery and by changing syringes, enough samples were obtained to indicate the adaptive changes in the blood. These are presented in figure 6. The climax was reached at the end of the three-minute period on 5.2 per cent oxygen. Arterial saturation was then down to 40 per cent, the pH had risen to 7.6 and the pCO_2 had dropped to 23 mm. Hg. During the 3 minutes on the 6.0 per cent mixture the oxygen saturation remained constant, but the pH declined and the pCO_2 increased. The first stage of recovery was accomplished with 14 per cent oxygen. This was uneventful.

TABLE 4

Composition of arterial blood in the deepest observed anoxia

DATE	SUBJECT	EXPOSURE		TOTAL CO_2 CONTENT	CO_2 COMBINING CAPACITY	pCO_2	pHg	O_2 SATURATION
1941		*min.*	*per cent O_2*	*vols. per cent*	*vols. per cent*	*mm. Hg*		*per cent*
July 16	B. H. E.	8.8	5.2	43.0	47.1	26.7	7.56	46
24	C. J. G.	11.5	4.2	41.9	45.9	24.2	7.58	43
22	S. J. A.	10.1	5.2	40.0	44.0	23.8	7.57	35
29	C. W. A.	8.9	4.2	37.9	48.0	20.2	7.53	41
21	D. F. W.	15.9	5.2	38.7	44.8	24.7	7.55	74
23	H. C. N.	7.2	4.2	41.5	42.2	31.3	7.45	46
21	M. D. R.	19.9	5.2	45.6	48.8	28.5	7.55	59
23	P. F.	7.4	4.2	43.4	43.6	32.5	7.45	51
22	Q. B. J.	10.0	5.2	46.8	49.3	29.1	7.51	50
17	C. J. H.	14.5	5.2	41.8	44.5	27.5	7.54	45

Clinical observations on the response of patients to 4.2 per cent oxygen are in brief as follows:

Cyanosis becomes quite noticeable in approximately 2 minutes; the ears are extraordinarily responsive. The hands get cold and clammy. At about the same time the patients seem to become sleepy. There is an obvious increase in the rate and depth of breathing. Perspiration becomes evident, especially in the axillae and the general thoracic areas.

Breathing becomes more difficult—sometimes gasping in nature. At this point the subjects frequently exhibit restless, unco-ordinated muscular movements, such as twitching of the mouth, chin, fingers, head, and even entire limbs. Saliva and mucus may accumulate and exaggerate the respiratory difficulties.

Sometimes the patients appear to hang on the verge of unconsciousness for several minutes. Their responses to commands become poor and answers to questions regarding date, age and birthday, that had been previously answered correctly, are frequently wrong. Repeating a question several times may elicit

a correct response even at the borderline of unconsciousness. At this point a few of the patients attempted to pull off their masks.

Unconsciousness, as evidenced by failure to respond to simple commands and the disappearance of the wink reflex, and in a few cases of the corneal reflex, comes on rather suddenly. In most cases this is so rapid as to give no preliminary warning. During the period of unconsciousness some become quite rigid with clonic movements. In two cases we observed spasmodic convulsions lasting respectively 15 and 60 seconds. These patients quieted down as the period of unconsciousness continued.

One patient commonly showed extensive and intensive muscular activity with rapid pulse and respiration before the anoxia was induced. After two minutes' exposure to low oxygen, he quieted down and remained so for some 5 to 10 minutes following completion of the test. In several other cases we also noted lessening of activity in the 5-minute recovery period. This may be associated with relief from the anxiety state into which the patient was thrown by the test.

On being returned to air or to 14 per cent oxygen, recovery is remarkably rapid, usually being well advanced within fifteen seconds. The ability to respond adequately to questions and commands is also restored rapidly. Cyanosis usually disappears within one minute. All effects are temporary in nature and leave the patient with neither ill effects nor recollections of the event.

Discussion. The therapeutic significance of these data will be presented in more detail elsewhere. It will suffice to say here that the mental condition of the patients was not changed. They were not benefitted by the procedures used nor, on the other hand, was there any evidence of damage to the central nervous system.

These patients revealed an unexpectedly high resistance to low oxygen mixtures and it seemed possible that loss of consciousness might occur at a higher level of arterial saturation if they had been in the erect or semi-erect posture. A few tests were made of this possibility by having the patient either standing or suspended in a harness such as forms a part of a parachute. The results were not different. This implies that in the stress of extreme anoxia not only the respiratory responses, but also the circulatory responses are strong and sustained; otherwise the cerebral circulation would reflect the changes in posture: consciousness would be lost earlier in the erect than in the reclining position.

These findings possess military significance because of their bearing on parachute escape. They indicate that flight personnel will not suffer permanent ill effects from oxygen lack if an escape is made at an altitude equivalent to a gas mixture containing 4.2 per cent oxygen.

The conversion of the percentages of oxygen used into equivalent altitudes is not simple. It cannot be accomplished by assuming that a given reduction in air pressure corresponds to a proportionate reduction in percentage of oxygen, the total pressure remaining constant, although this mistaken idea has persisted in the literature since the last war (4, 5). As a result of this error unreliable deductions have been drawn from observations of anoxia induced by air-nitrogen

mixtures or by a re-breather of the closed circuit type in which carbon dioxide is absorbed.

The usual method of calculation is based on the assumption that a given partial pressure of oxygen produces like physiological effects regardless of the total pressure. Thus it is commonly stated that 10.5 per cent oxygen at 760 mm. Hg is equivalent to 21 per cent oxygen at 380 mm. Hg. If the diluting effects of water vapor and of carbon dioxide are taken into account, it appears that 10.5 per cent oxygen is actually equivalent to a higher barometric pressure than 380 mm. Hg.

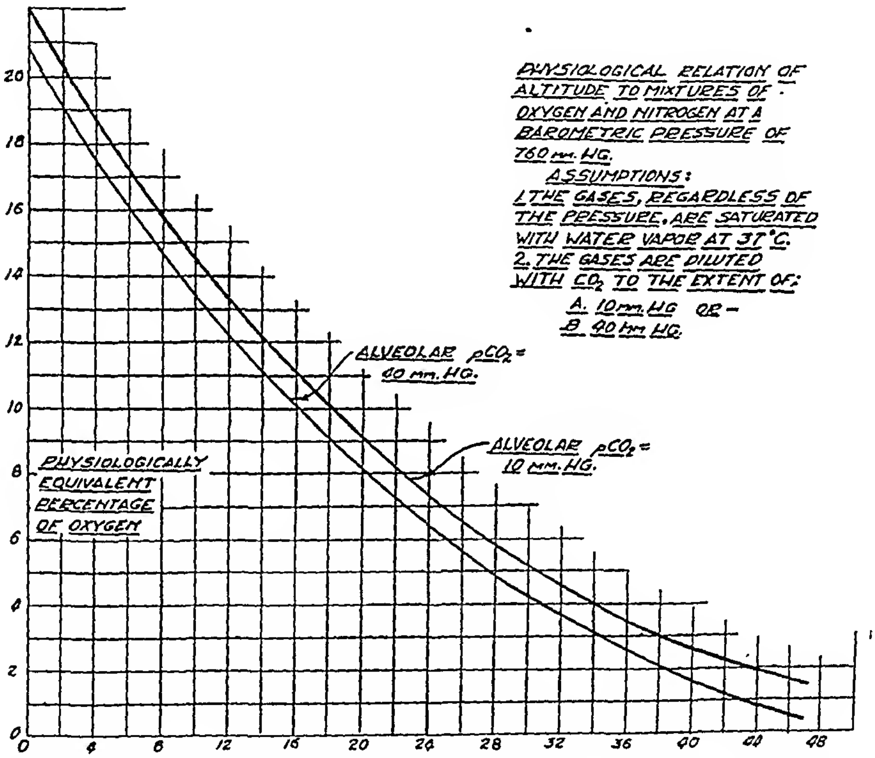

Fig. 7. Physiological relation of altitude to mixtures of oxygen and nitrogen inspired at sea level. Altitude is in thousands of feet.

A sample calculation is as follows:

It is assumed that the air as it is drawn into the lungs is warmed to 37°, saturated with water vapor up to pH_2O of 47 mm. Hg and admixed with enough carbon dioxide to yield a partial pressure of 40 mm. Hg. Hence, without allowing for the uptake of oxygen by the lungs, the partial pressures of oxygen (pO_2) in the two instances will be:

$$pO_2 = (760-47-40)0.105 = 71.7$$
$$= (380-47-40)0.21 = 61.5$$

It follows as will be seen below, that 10.5 per cent oxygen at ground level is equivalent to about 17,000 feet rather than 18,000 feet as is commonly stated. One consideration has not been taken into account in the foregoing calculation:

as carbon dioxide is being added, oxygen is being removed. The state of gas exchange in the lungs is one of dynamic equilibrium complicated by *a*, the constant movement of oxygen into the blood and of carbon dioxide out of the blood; *b*, by the rhythmic movement of air into and out of the lungs, and *c*, by the varying degrees of admixture of freshly inspired air with air in the depths of the lungs. As a first approximation, the method of calculation employed gives results in accord with empirical observations.

Equivalent altitudes have been calculated for two partial pressures of carbon dioxide. The results are shown graphically in the accompanying figure. If one accepts an intermediate pCO_2 of 30 mm. Hg as being characteristic of the tests made in this investigation, the altitudes may be set down as follows:

OXYGEN IN INSPIRED GAS	EQUIVALENT ALTITUDE
per cent	*feet*
4.2	31,000
5.2	28,000
6.0	26,000

These results have a military interest because of their bearing on the technic of parachute escape. In the absence of an accessory oxygen supply should one pull the rip cord at once if it is necessary to bail out at 31,000 feet? Romberg (6) felt that parachute descent from 36,000 feet, simulated in a low pressure chamber, was deleterious, being accompanied in all cases by collapse. He recommended a free fall until "safe" altitudes of 15,000 to 18,000 feet were reached. Since a man with an opened parachute will fall from 31,000 feet to 25,000 feet in about three minutes the tests reported herein indicate that the anoxia experienced while breathing air within this altitude range would have no harmful effects. Many individuals would not lose consciousness, and all those who do should fully recover consciousness long before ground level is reached.

SUMMARY

Schizophrenic patients have been subjected to severe anoxia over a period of several minutes either up to the point of unconsciousness or in some cases extending into unconsciousness. The following conclusions are drawn:

Anoxia of severe degree produces no beneficial effects on the mental condition of this class of psychotic patients.

Anoxia severe enough to produce brief periods of unconsciousness has no lasting harmful effects on the central nervous system.

Respiratory stimulation by anoxia is strong and sustained even during unconsciousness.

Inferentially, circulatory function is also well sustained.

There is a remarkably rapid return to normal when either air or 14 per cent oxygen is supplied.

A mixture of 4.2 per cent oxygen with nitrogen is equivalent physiologically to an altitude of about 31,000 feet.

It should be possible to descend with an opened parachute from 31,000 feet altitude without oxygen equipment with no ill effects from anoxia.

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AGE AND THE CALORIGENIC RESPONSE TO SUBCUTANEOUSLY ADMINISTERED ADRENALIN IN THE RAT

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Although the calorigenic response to subcutaneously administered adrenalin has been extensively studied in animals and man there is little to be found in regard to it in connection with the species most commonly used in metabolic work, the albino rat (Cori and Cori, 1928; Carr et al., 1934); and such information as there is describes only the total, average effect over $2\frac{1}{2}$ to 3 hour periods, with no information as to the time-course of the response.

The work to be described here was designed to fill this gap. In the course of it, evidence appeared that the character of the response altered significantly with age; this was therefore explored systematically with groups of animals from 2 to 28 months old.

METHODS. The animals used were male albino rats of our colony, a hardy, fertile, normal-growing Wistar strain inbred for generations and quite free from organic disease and internal or external parasites. They were reared and maintained on a diet of Purina Dog Chow in a room the temperature of which fluctuated only within the narrow range of 21 to 25°C.

Measurement of the respiratory metabolism was made with the apparatus described by Schwabe and Griffith (1938), modified for the CO_2 determination as described by Kingdon et al., (1942).

This apparatus is especially useful for the purpose here in mind since it provides continuous record of O_2 consumption and allows minute-to-minute measurement of CO_2 production. These are both indispensable for determination of the respiratory metabolism within short intervals and particularly for recognition and estimation of the values characteristic of the relatively short periods of quiescence on the part of the animal immediately after introduction into the chamber of the apparatus following the disturbing effect of an injection, and especially the injection of adrenalin.

In addition to initial, thorough calibration, the apparatus was subjected to frequent flame checks, by burning a tiny jet of illuminating gas at rates simulating the O_2 consumption and CO_2 production of the rat, after the manner of Bunnell and Griffith (1940).

During an experimental run the animal chamber of the apparatus was always at 28 to 29°C., the point of "thermic neutrality" as described by Benedict and MacLeod (1929).

DETERMINATIONS. A. BASAL. At 4 o'clock in the afternoon preceding a determination food was removed from the animal cage; the animal was then brought to the laboratory 17 to 18 hours later at 9 or 10 o'clock the next morning, placed in the chamber of the apparatus, and allowed as much time as necessary ($\frac{1}{2}$–$\frac{3}{4}$ of an hour) to settle down to a rigorously basal condition.

Following this basal determination the animal was removed from the apparatus and treated either as a control or with an injection of adrenalin as follows.

B. Controls. *1. Handling.* After the basal determination, as just described, the animals to be used for this purpose, five in number, were removed from the apparatus and handled as if to receive an injection. They were then replaced and determination of metabolic rate made as frequently as possible for the next four hours. Although the record consists only of data derived during intervals of no overt activity, these, especially during the first half-hour or so, after return to the chamber, were of short duration and not likely to have been preceded by a sufficiently long rest period to be considered strictly basal. But since, as will be seen, it was equally or even more impossible to secure rigorously basal determinations within the first hour or so after injection of adrenalin, this method of procedure was all the more necessary for proper evaluation of the immediate, specific action of adrenalin itself. After this preliminary period following return to the chamber, and whether merely handled or injected, the animal settled down sufficiently so that most of the determinations from then on approximate basal values.

2. Saline injection. Following the preliminary basal determination the animals used for this purpose, three in number, were removed from the apparatus and injected subcutaneously with 0.9 per cent NaCl in amount equal to that serving as vehicle for the injected adrenalin as described below. They were then immediately replaced in the chamber of the apparatus and determinations made as frequently as possible and whenever there was no overt activity during the following hour. As mentioned under the previous heading these, for the most part, could not be strictly basal, but corresponded even more closely to those obtainable immediately after injection of adrenalin and are, therefore, particularly useful in evaluation of its effect.

C. Adrenalin injection. Again, this followed a preliminary basal determination, the animals, thirty-three in number, then being removed from, injected and returned to the apparatus with as little delay as possible and the metabolic rate followed as continuously as could be done with avoidance of periods of active movement, for the following four hours.

For the first hour or so after this injection the animal was particularly unquiet with a type of restlessness qualitatively different from the mere occasional bodily movement shown by the controls and characterized by panting, fine tremor (Choi, 1928; Hartman et al., 1927, 1928, 1929) and lying on the back. These and other activities subsided toward the end of the first hour after injection so that from then on the determinations again approach basal values. Such, however, are impossible to obtain during the early part of the action of adrenalin in the dosage employed here.

Following precedent (Cori and Cori; Carr et al.), the dose used was 0.02 mgm. per 100 grams body weight. This was injected with the necessary amount of 0.9 per cent NaCl (0.1 cc. of 1:1000 Parke-Davis adrenalin chloride solution was diluted to 1 cc. with salt solution and the requisite amount of this used for injection) subcutaneously on the medial aspect of the thigh.

RESULTS. *Controls.* Figures 2 and 3 show the extent to which measurement of the respiratory metabolism is affected by handling, control saline injection and the effort to secure determinations immediately after placement of the animal in the chamber of the apparatus and before there has been time for it to quiet down to a truly basal condition.

Oxygen consumption (fig. 2) is apparently unaffected to any significant degree by these experimental procedures. Considering that the beginning of these records coincides with about the 18th or 19th hour of fasting, the continuous slight decline of the record for the handled rats for the 4-hour period shown in the graph is understandable as the effect of prolonged fasting time. The absence of any noticeable elevation of rate above basal during the first hour of the record, when the animals were most restless shows that as long as measurements are confined to

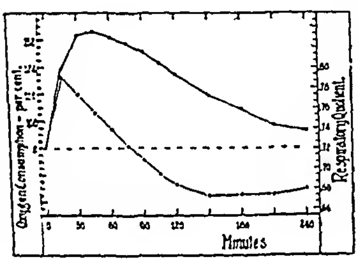

Fig. 1

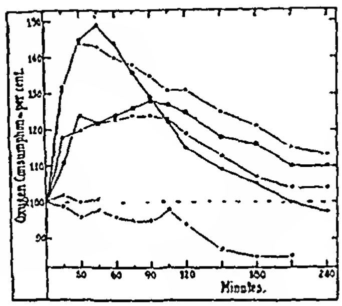

Fig. 2

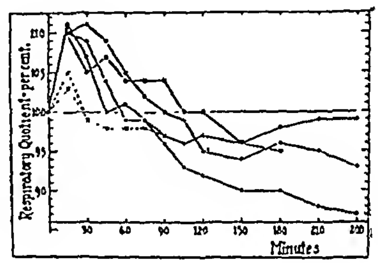

Fig. 3

Fig. 1. Upper curve, oxygen consumption (per cent of the preliminary basal value) and lower curve, respiratory quotient (actual values) for four hours following subcutaneous injection of 0.02 mgm. per 100 grams body weight; average responses of 33 male albino rats varying in age from 2 to 28 months.

Fig. 2. Oxygen consumption (per cent of preliminary basal) of controls and following the injection of adrenalin (dose as in fig. 1) at different ages:

x —— x ——, controls. Upper curve, saline injected (3 animals); lower curve, handled only (5 animals).

○ —— ○ ——: 2–6 months of age (9 animals).

● —— ● ——: 12–18 months of age (8 animals).

+ —— + ——: 19–24 months of age (9 animals).

□ —— □ ——: 25–28 months of age (7 animals).

Fig. 3. Respiratory quotient (per cent of preliminary basal) of controls and following the injection of adrenalin (dose as in fig. 1) at different ages; the different curves as in figure 2.

the even, short periods in which there was no overt movement these are not significantly affected by activity of the degree here encountered though it immediately preceded the determination.

Respiratory quotient (fig. 3) is more noticeably affected, especially during the first half-hour after replacing the animal in the apparatus. During this time it is raised temporarily 3–5 per cent above the previously determined basal, indicating that the restlessness characteristic of this early period before the animal settles down sufficiently to make strictly basal determinations possible does have a carry-over effect on CO_2 output during such brief periods of inactivity as occur and are measurable.

Adrenalin: A. Average effect. Figure 1 describes the total average effect on oxygen consumption and respiratory quotient for four hours following the administration of adrenalin. Each curve is the average of 33 complete, four-hour determinations.

Oxygen consumption will be seen to reach a maximum, 34.5 per cent above the preliminary normal basal value, 45 minutes after injection. From then on there is almost uniform decline which does not quite reach the initial basal value at the end of the four hour period. The actual figures in percentage above normal, for each of the subsequent time intervals shown on the curve are: 60 min., 33.1; 75 min., 31.1; 90 min., 28.9; 105 min., 25.5; 120 min., 22.1; 150 min., 15.9; 180 min., 11.8; 210 min., 6.8; 240 min., 5.6. Actually, if as probably should be done, the four-hour control curve of figure 2 were made the basis of estimate throughout, rather than the initial preliminary basal determination, the increase due to adrenalin would be greater at all time intervals and the elevation above the expected normal rate (reduced by prolonged fasting time) at the end of the four-hour period, approximately 20, rather than 5 per cent. Evidently, then, adrenalin is either still being actively absorbed four hours after injection (Cannon, 1929; Cori, 1929) or the metabolic disturbances initiated by it (Cori, 1931) are still responsible for a maintained elevation of O_2 consumption.

How much of the initial, peak-rise in oxygen consumption is attributable to the restlessness caused by this dosage of adrenalin cannot be estimated with complete certainty. It cannot be stressed too strongly that basal values, in the accepted sense of determinations made while the animal is quiet and after it has been so for a sufficiently long preliminary period, are impossible to obtain for an hour or so after injection of the amount of adrenalin used here. One can either measure the total metabolic rate as has been done by others; or as has been done here, and with an apparatus which we believe is sensitive enough to do so with some accuracy, confine the measurement to those short intervals in which the animal is not actually moving about, although it has been doing so immediately before. The control curves of figure 2 indicate that this can apparently be done with some success in the case of normal animals; and that the amount of activity resulting from preliminary handling, control saline injection, and recent introduction into the apparatus does not prevent measurements confined to short, quiet intervals from approximating very closely the actual basal value. To this extent there can be confidence that the adrenalin effect as we have measured it is not largely attributable to overt activity. The fine muscular tremor and panting induced by this amount of adrenalin cannot, however, be evaluated by control experiments; indeed, as an apparently inevitable and integral part of the action of adrenalin (in this amount) it would probably be unwise to attempt the exclusion of their effect from measurement of the total metabolic response to adrenalin as we are interested in it here.

From the beginning of the second hour these disturbing factors progressively lessen and the determinations from then on approach more and more closely truly basal values.

The total increase in O_2 consumption for the 4-hour period, as obtained by

rough integration of the area beneath the curve of figure 1, amounts to 19.5 per cent. This agrees closely with the increase of approximately 17 per cent obtained by the Coris (1929) for the first 3 hours, but is considerably less than the 32 per cent increase reported by Carr et al. (1934) as the total increase for the first $2\frac{1}{2}$ hours following injection. In fact, in both of these instances values higher than ours should be expected since they apply only to the earlier, more markedly increased part of the response and the methods used measured the total rate of metabolism and did not admit of any attempt to eliminate periods of overt bodily movement.

Respiratory quotient is also seen from figure 1 to increase sharply within the first 15 minutes following injection, from an average basal value of 0.72 to a maximum of 0.79. Thereafter it falls progressively, reaching normal again within 75 minutes, and continuing to decrease to a minimum of slightly below 0.68, which is held between the 150th and 210th minutes; within the final half-hour there is apparently the beginning of a return toward normal.

The initial peak increase apparently is due in considerably greater part in this case than in that of O_2 consumption to the experimental procedures of handling, injection and immediate introduction of the animal into the apparatus. As shown by the control curves of figure 3, these are associated with a definite increase (to 0.742 and 0.757, respectively) in the respiratory quotient during the initial half-hour. The difference, however, between the controls and adrenalin-injected animals is considerable and shows that most of the increase during this time and until the end of 75 minutes is due to adrenalin. What part of this is a specific effect on the nature of the foodstuffs burned and what is indirect and related to the acidosis (lactacidemia) and excitement, with increased pulmonary ventilation and "Auspumpung" (Griffith et al., 1939, 1940) cannot be completely decided from the evidence at hand. The latter, however, would seem to be especially implicated since the subsequent depression below normal almost entirely compensates for this initial rise; the total respiratory quotient for the entire four-hour period, 0.71, as obtained by rough integration of the curve of figure 1 is, as was previously noted, also, by the Coris, and Carr, et al., practically unchanged from the preliminary normal basal value.

Adrenalin. B. The effect of age on the response. As mentioned in the introduction, it seemed evident from some of the early experiments which were done on animals of quite different ages, that this was responsible for an alteration in the nature of the response. This was thereafter taken into consideration and the work extended to include animals from 2 to 28 months old. The results are presented in figures 2 and 3, for which purpose the total number of 33 animals has been arbitrarily divided into four groups of approximately equal number (2–6 months of age, 9 animals; 12–18 months, 8 animals; 19–24 months, 9 animals; 25–28 months, 7 animals) in order to secure average curves of comparable validity.

Oxygen consumption. It is apparent from figure 2 that the response of animals less than 18 months of age differs markedly during the first $1\frac{1}{2}$ hours from that of those which are older. Both of the younger groups show a sharp and prompt increase to a maximum (45 and 49 per cent) within 30 or 45 minutes after injec-

tion. In those animals 19 months of age and older this peak is entirely absent, the O_2 consumption rising only slowly to a maximum (24 and 28 per cent) about 90 minutes after injection. At this time the increase is about the same in all age groups and hereafter the O_2 consumption returns toward normal at an approximately equal rate for all such differences as are shown by the different groups during this later portion of the response probably being due merely to insufficient evidence.

It seems improbable, however, that mere lack of data can be responsible for the apparently pronounced difference in the earlier part of the response, although more extensive evidence is probably needed even here to describe the difference with real accuracy. It would seem improbable, for example, that the transition from one type of response to the other should be as abrupt as these results indicate. More likely, an adequate amount of data month-by-month would provide evidence of a more gradual transition; indeed, something of the sort seems to be indicated even by these meagre data: the maximum peak-response is shown by the youngest group of rats; the next older group shows a slight reduction toward the type of response typical of the oldest groups.

The only explanation that suggests itself for this difference is a slower absorption of adrenalin from the subcutaneous depot in the older animals. Although this assumption does not seem too improbable in light of the known ageing of the vascular system (Cohn, 1939) we know of no direct evidence in favor of it.

Respiratory quotient is not affected in the same way as oxygen consumption, although here, too, age does seem to alter the response. It will be seen from figure 3 that the maximum peak increase is practically the same in all age groups, both as to magnitude (10–11 per cent) and time of occurrence (15–30 min.) after injection. What difference there is seems to lie in the length of time the respiratory quotient is elevated above normal; this is least for the youngest age group and greatest in the oldest. Although it is not easy to see how maximum intensity could be unaffected by varying rate of adrenalin absorption, a delay of this with age would account for the prolongation of the increased quotient and its delayed return to normal.

Any further speculation as to the cause of these apparently different results at different ages would be premature until the fact is better established by more abundant evidence. These data are only sufficient to arouse interest in the possibility that a difference does exist.

SUMMARY

Subcutaneous injection of adrenalin (0.02 mgm. per 100 grams body weight) in male albino rats, 2 to 28 months old, causes an average maximal increase in O_2 consumption of 34.5 per cent within 45 minutes after injection; thereafter there is gradual decline to a value still about 5 per cent above the preliminary basal figure four hours after injection. Respiratory quotient increases from an average basal value of 0.72 to 0.79 within 15 minutes after injection; from this maximum it declines to normal within 75 minutes and continues to decrease to approximately 0.67 which is reached within 150 minutes and is maintained with only

slight tendency toward recovery until the end of the four-hour period. The total average increase in O_2 consumption for the four-hour period is 19.5 per cent; the preliminary increase in respiratory quotient is so nearly balanced by the compensatory fall that the total value for the four-hour period is practically unchanged from the preliminary normal.

In the older rats the maximum increase in oxygen consumption is less and is delayed until 90 minutes after injection; the maximal increase in respiratory quotient is practically the same and is reached at about the same time in all age groups, but the return to normal seems significantly delayed in proportion to increasing age. A delay in the absorption of the subcutaneously injected adrenalin proportional to age is tentatively suggested as a possible cause of these effects.

The increase in respiratory metabolism during the first hour or so after injection of this standard dose of adrenalin is undoubtedly due in part to the restlessness which it causes. The values reported here are probably not greatly affected by overt bodily movement, but it is impossible to eliminate the effect of the persistent muscular tremor which is characteristic of this initial period of action of this amount of the hormone.

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THE EFFECT OF DIETARY COMPOSITION ON PANCREATIC ENZYMES

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The ability of the pancreas to alter its external secretion by increasing the output of any given enzyme or enzymes in relation to the predominance of any particular substrate in the diet is a question which has long been debated and never adequately decided. Many years ago it was stated without the submission of adequate evidence that the pancreatic enzymic secretory response to a single meal was adapted to the type of food (1)—e.g., a meat meal elicited a secretion higher in tryptic activity than a meal low in protein.

Today it is generally believed that variations in the principal pancreatic enzymes take place in a parallel manner; in other words, a rise or fall in any one enzyme is accompanied by a coincident change in each of the others. For example, cholinergic drugs elicit a concentrated secretion rich in enzymes, whereas secretin provokes the formation of a dilute juice of low enzyme content; but in any given animal the ratio of concentration of the individual enzymes is the same, regardless of the type of stimulus. Likewise, the pancreatic juice formed in response to various meals may vary in concentration, but not in the relative amounts of the several enzymes. This belief may be true; yet it is still possible that, for example, a diet high in protein after being fed for several days may call forth a secretion richer in trypsin.

The parallelism of enzyme concentrations in the course of acute experiments has been established for a number of species, including the dog (2–4), rabbit (5), cat (6), and human (7). However, the ratio of the concentrations of the various enzymes shows marked variation, both from animal to animal and in the same animal over considerable periods of time. It is not inconceivable that such changes are the result of adaptation to a pronounced and prolonged change in the diet.

This possibility has been examined and never adequately confirmed. Two reports from Pavlov's laboratory (8, 9) state that in pancreatic fistula dogs the protease content increased on a meat diet and subsequently fell off on a high carbohydrate regime. In each case data were apparently obtained on only one animal and the findings were never eonfirmed. Later investigators devoted their attention to determinations of active and inactive enzymes on the various diets, with inconclusive results (10). More recently the enzyme content of the duodenal drainage of human subjects on various diets has been found to vary in relation to the predominating type of foodstuff in the diet (11) with noticeable differences detectable within a few days.

That this problem has not been adequately settled in the past is due to a

number of causes, including *a*, the variability in secretion of pancreatic fistula dogs, which would require sufficient data for statistical analysis; *b*, the inadequate number of control animals and control periods; *c*, inaccurate dietary control; *d*, insufficient duration of experimental observation; *e*, inaccurate methods for enzyme analysis; and *f*, in the case of duodenal drainage fluid, the contaminated condition of the pancreatic juice.

A technique calculated to overcome these difficulties has been devised by us, which depends for its effectiveness on the principle that the concentration ratio of enzymes in pancreatic juice is the same as that existing in the gland which secretes it. The accuracy of this conception was established by appropriate analyses of pancreatic secretion and pancreatic tissue in a series of dogs, and thus the necessity of fistula animals was eliminated. A number of diets of varying compositions was then fed to white rats for a requisite interval, at the conclusion of which the enzyme content of the pancreatic tissue was determined.

Experimental. The methods for enzyme analysis were those successfully employed in this laboratory for some time. Lipase was determined by the method of Cherry and Crandall (12), amylase by the Willstätter (13) procedure as adapted by us (14), and trypsin by a colorimetric method devised after that of Northrop (15). The results are expressed in the tables below as follows: lipase in cubic centimeters of 0.05 N NaOH required to neutralize the fatty acids liberated from an olive oil emulsion substrate, amylase in milligrams of maltose liberated from a starch substrate, and trypsin in terms of milligrams of tyrosine liberated from a casein substrate.

The enzyme content of pancreatic tissue was determined on weighed amounts of the dried gland. The pancreas was removed immediately after death of the animal, macerated in a mortar, and suspended in cold (15°C.) acetone for 3 hours. The acetone was removed by centrifuging and the tissue resuspended in fresh acetone for 10 minutes at room temperature, collected by centrifuging, washed with dry ether, and finally air-dried, pulverized, and stored in stoppered glass containers. Weighed amounts of the powdered gland were suspended in 1/15 molar phosphate buffer at a pH of 7 for one hour at room temperature in the proportion of 0.2 or 0.3 cc. per milligram, the mixture filtered, and the enzyme content of an aliquot of the filtrate determined.

In the series of dogs employed for purposes of comparison of enzyme content in pancreatic secretion and in the gland itself, the procedure involved anesthetization of the animal with sodium pentobarbital, cannulation of the main pancreatic duct, and collection of the secretion after stimulation with an adequate amount of secretin. The pancreas was then excised and treated as indicated above.

One hundred sixty-two young white rats (100–150 grams) served as experimental animals for the study of adjustment of pancreatic enzymes to the type of food administered. The diets had the composition shown in tables 1 and 1A and were fed *ad libitum* for a period of 21 days, at the completion of which the animals were sacrificed by cervical fracture, and the pancreas promptly and completely dissected out, and treated as above for enzyme determinations.

Most of the animals were maintained on isocaloric diets (table 1A), in order to make certain that all essential components were ingested in amounts exceeding the minimum requirement, and thus avoid complications which might arise from any deficiency.

RESULTS. 1. *Comparison of enzyme content of pancreatic juice and of dried pancreas.* It was found in the ten animals (anesthetized dogs) tested that in each case there was a parallelism between the concentration of the three chief pancreatic enzymes present in the gland itself and in the juice secreted by it. The results in detail are listed in table 2.

TABLE 1

Composition of high carbohydrate, high protein and high fat diets

	DIET A HIGH CHO	DIET B HIGH CHO	DIET C HIGH PROTEIN	DIET D HIGH PROTEIN	DIET E HIGH FAT
Casein	18	10	60	65	10
Dextrin	47	55	0	0	0
Sucrose	13	13	13	13	13
Salt mixture (Osborne-Mendel)	4	4	4	4	4
Lard	5	5	10	5	60
Agar	2	2	2	2	2
Fish liver oil	3	3	3	3.	3
Yeast	8	8	8	8	8

TABLE 1A

Isocaloric diets

	DIET H BALANCED			DIET I HIGH CHO			DIET G HIGH PROTEIN			DIET F HIGH FAT		
	Wt.	Cal.*	Cal.	Wt.	Cal.	Cal.	Wt.	Cal.	Cal.	Wt.	Cal.	Cal.
	per cent		*per cent*	*per cent*		*per cent*	*per cent*		*per cent*	*per cent*		*per cent*
Casein	18	72	15	15	60	15	67	268	65	25	100	15
Starch	47	188	38	67	268	65	15	60	15	0	0	0
Salt	4	0	0	4	0	0	4	0	0	4	0	0
Cellulose	2	0	0	2	0	0	2	0	0	2	0	0
Lard	18	162	33	3	27	6	3	27	6	54	486	72
Yeast	8	40	8	6.5	33	8	6.5	33	8	11	55	8
Fish liver oil	3	27	5	2.5	23	5	2.5	23	5	4	36	5
Totals	100	489	99	100	411	99	100	411	99	100	677	100

* Calories.

2. *Enzyme composition of pancreatic tissue of rats on constant diets.* When the enzyme values for the various diets are compared with those of diet H, the balanced diet, a number of differences are observed. The series of animals fed the high carbohydrate diet (diets A, B and I) showed a marked predominance of

amylase in the pancreatic tissue. There was a repression of trypsin, and the lipase value was unchanged. Likewise, there was an increased trypsin content in the rats fed the high protein diet (diets C, D and G). On the high fat regime (diets E and F) there was repression of amylase, while the lipase and trypsin

TABLE 2

Enzyme composition of 100 mgm. of pancreatic extract and 0.02 cc. of pancreatic juice in the same animal

DOG NO.	SOURCE	AMYLASE		TRYPSIN		LIPASE	
		Maltose	Ratio extract to juice	Tyrosine	Ratio extract to juice	NaOH	Ratio extract to juice
		mgm.		*mgm.*		*cc.*	
1	Extract Juice	420 950	0.44	9.32 19.9	0.47	156 355	0.44
2	Extract Juice	390 675	0.58	6.32 8.15	0.78	132 195	0.68
3	Extract Juice	434 615	0.71	13.6 20.1	0.68	202 290	0.70
4	Extract Juice	360 770	0.47	9.88 20.4	0.48	168 350	0.48
5	Extract Juice	292 550	0.53	11.6 23.9	0.49	126 240	0.52
6	Extract Juice	286 642	0.45	10.3 25.8	0.40	242 494	0.49
7	Extract Juice	362 488	0.74	12.8 15.6	0.82	298 354	0.84
8	Extract Juice	435 572	0.76	21.4 27.1	0.79	308 380	0.81
9	Extract Juice	392 478	0.82	17.4 21.2	0 81	281 334	0.84
10	Extract Juice	414 600	0.69	29.8 48.8	0.61	386 665	0.58

values remained essentially unaltered. All enzymes were repressed on a high fat diet containing 10 per cent protein (diet E); the 24 animals in this series had fatty livers and small atrophic pancreases. Addition of 1 per cent of choline to the diet resulted in an increase in all the pancreatic enzymes to approximately the same degree, and a normal histological picture of the liver and pancreas.

The detailed data for all animals and the average values are listed in table 3.

DISCUSSION. It is apparent on the basis of the data submitted that the type of diet ingested influences the enzyme make-up of the pancreas and its secretion. Thus the 39 rats on a high carbohydrate diet developed very high amylase values, with a repression of trypsin and essentially unchanged lipase. The series on a high protein intake had a high trypsin content of the pancreas; the lipase values were also highest on this regime, while the amylase was repressed. In the case of the high fat diet there was a repression of amylase, with essentially unaltered values of trypsin and lipase. The significance of the changes in composition was demonstrated by statistical analysis (table 4).

The most marked responses to dietary modifications are elevated amylase and depressed trypsin values in the case of a high carbohydrate diet, elevated

TABLE 3

Average enzyme content of pancreatic tissue of rats

NO. OF RATS	RAT NUMBERS	SERIES	DIET	DESCRIPTION	AMYLASE		TRYPSIN		LIPASE	
					Range	Ave.	Range	Ave.	Range	Ave.
4	1–4	I	A	High CHO	370–380	376	4.4– 8.0	5.5	74–94	86
10	13–22	II	B	High CHO	291–465	336	0.6– 33.0	9.2	309–519	406
25	118–142	IV	I	High CHO	546–1140	689	1.5– 17.4	9.1	216–423	333
4	5–8	I	C	High protein	152–304	225	4.4– 49.6	20.8	100–154	128
10	23–32	II	D	High protein	219–278	252	26.7– 68.4	48.2	360–660	556
25	68–92	IV	G	High protein	165–540	398	16.5–103.5	60.9	321–555	464
4	9–12	I	E	High fat	142–206	174	1.0– 29.6	9.4	64–88	75
10	33–42	II	E	High fat	9–282	147	8.1– 41.4	24.8	150–540	342
10	143–152	III	E	High fat	48–312	171	1.8– 12.9	5.8	51–123	83
10	153–162	III	E*	High fat plus 1% choline	108–462	309	9.6– 34.2	20.7	87–324	184
25	43–67	IV	F	High fat	60–264	170	3.0– 44.4	24.4	60–564	316
25	93–117	IV	H	Balanced	285–606	489	2.4– 73.5	20.3	192–495	358

E* = Diet E plus 1% choline.

trypsin on a high protein regime, and depressed amylase on a high fat intake. No marked alterations in lipase occurred on any of the diets, save for a definite increase when ½ of the diet was supplied by protein. These experiments, therefore, bear out in part the long-standing hypothesis that the composition of the pancreatic juice is governed by the type of foodstuffs ingested. The mechanism whereby this takes place is at present obscure, and its explanation must await an elucidation of the process of formation of the pancreatic enzymes. Whether this takes place on a metabolic, neurogenic, or other basis is at present unknown. The situation is somewhat analogous to that of some bacteria, which are known to produce certain enzymes in response to the predominance of a given substrate in the diet (16).

The animals on the high fat-low protein diet (diet E) manifested a depression of all enzymes in their pancreatic tissue. These animals remained in good

condition throughout the experiment, except for a shaggy and greasy appearance of the coat of hair. On autopsy the pancreas was small and atrophic, with indications of parenchymatous degeneration in the acinar tissue, while the islet tissue was normal in appearance, and the liver was very fatty. Addition of 1 per cent of choline to this diet (diet E) resulted in a uniform increase in all the enzymes determined (table 3). On this regime the liver and pancreas showed no histological changes.

As a matter of physiological economy, it might be expected that the enzyme output of a digestive gland should be subject to modification in accordance with the predominating foodstuffs present in the diet, in order that the organism may utilize its available materials with a maximum of efficiency. Such changes in composition as are brought about by any given dietary regime undoubtedly occur in the gland itself, and apparently the external secretion of the pancreas represents an aqueous extract of the organic material in the acinar tissue. This is borne out by the parallelism in enzyme content of the secreted juice and the dried pancreas in the ten dogs studied; and it is on the validity of this assumption that the accuracy of the interpretation of the data reported is based.

TABLE 4

Statistical analysis of enzyme data on rat 43-142, indicating significance of values observed

DIET	MEAN	STANDARD ERROR OF MEAN	DIFFERENCE OF THE MEANS	STANDARD ERROR OF DIFFERENCE OF MEANS	CRITICAL RATIO "C"	PROBABILITY OF OCCURRENCE OF A DEVIATION AS GREAT AS DESIGNATED "C"
			Amylase			
CHO	689	±23.3	200	±28.9	6.9	0.26×10^{-11} %
Bal.	489	±17.4	91	±31.9	2.9	0.373%
Prot.	398	±26.7	128	±28.9	4.4	0.003%
Fat	170	±11.0				
			Trypsin			
Prot.	60.9	±4.5	36.5	±5.1	7.2	$.26 \times 10^{-11}$%
Fat	24.4	±2.4	4.1*	±4.3	0.95	33%
Bal.	20.3	±3.6	11.2	±3.7	3.0	0.27%
CHO	9.1	±0.99				
			Lipase			
Prot.	464	±11.6	106	±18.2	5.8	2×10^{-5}%
Bal.	358	±14.0	25*	±16.8	1.5	13%
CHO	333	±9.3	17*	±27.3	0.6	50%
Fat	316	±25.6				

* These differences not significant.

SUMMARY AND CONCLUSIONS

1. In rats maintained for three weeks on a constant diet, an adaptation of the composition of chief pancreatic enzymes to the predominant constituent of the

diet was noted. Thus on a high carbohydrate diet there was a pronounced increase in amylase, together with a decrease in trypsin. A high protein diet resulted in greatly increased trypsin content and a less extensive, but definite, increase in lipase. On a high fat diet there were essentially no alterations in lipase or trypsin.

2. A diet which is high in fat and low in protein causes a repression of all pancreatic enzyme formation. The addition of 1 per cent choline to such a diet increases uniformly the content of all enzymes.

3. The enzyme content of pancreatic juice parallels that existing in pancreatic tissue.

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THE EFFECT OF ATROPINE ON THE CORONARY BLOOD FLOW OF TRAINED DOGS WITH DENERVATED AND PARTIALLY DENERVATED HEARTS

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In previous studies (1, 2) from this laboratory it has been shown that intravenous injections of atropine sulfate result in prolonged increases of coronary blood flow and heart rate in trained dogs with innervated hearts. Similar results were obtained on animals under chloralosane anesthesia. In addition it was found that changes of the arterial blood pressure following the administration of atropine sulfate were insignificant; therefore the augmented coronary blood flow could not result from a change of blood pressure. The possibility that the increased heart rate might be at least partially responsible for the greater coronary blood flow was explored by Hausner and others (3) using the so-called denervated heart-lung preparation. In that study it was found that increases and decreases of heart rate produced by means of Hill's stimulator caused, respectively, increases and decreases of the coronary blood flow. The absence of significant changes of blood pressure following injections of atropine left the augmented heart rate and certain nervous influences as possibly responsible for the increased coronary flow. It was also necessary to consider the possibility that atropine affected directly the walls of the coronary blood vessels.

In another investigation we were observing the effect of exercise on the denervated and partially denervated heart of the trained dog (4). The observations reported here were made on the animals used in that study.

METHODS. In the present study the response of the heart rate and the coronary blood flow to injections of atropine sulfate (0.1 to 0.2 mgm. per kgm.) was observed in dogs after the following procedures: 1, removal of both sympathetic chains of ganglia from the eighth or ninth intercostal space including the stellate ganglion; 2, double cervical vagotomy, and 3, a combination of procedures 1 and 2. The operations were done employing sterile technic and with the animals under general anesthesia. The section of the left vagus was usually done under infiltration anesthesia with procaine hydrochloride. The coronary blood flow was measured by means of the thermostromuhr and the units were applied at varying intervals after the operative procedures indicated under 1, 2 and 3. An effort was made to make all observations before sufficient time had passed for regeneration of the nerves to have occurred. The blood pressure was recorded optically from a cannulated femoral or carotid artery.

RESULTS. Although a considerable series of observations was made the results were sufficiently uniformly positive that only a few representative experiments will be described in detail.

Effects of atropine after sympathetic ganglionectomy. In the absence of the cardiac sympathetic nerves, injections of atropine caused a marked increase of heart rate and an augmented coronary flow of 25 to 100 per cent. The effect of the drug was evident in twenty to thirty seconds after injection. The blood pressure was not elevated significantly in any of the experiments. In the experiment represented in figure 1 the maximal change of coronary flow was nearly 100 per cent. The heart rate rose from 70 to 200 beats each minute. The rectal temperature was 102.1°F. The mean blood pressure taken by means of a glass spoon manometer (5) was practically unchanged for three minutes, when a few fluctuations occurred in the next two minutes. Thereafter, however, the blood pressure remained relatively constant. The coronary blood

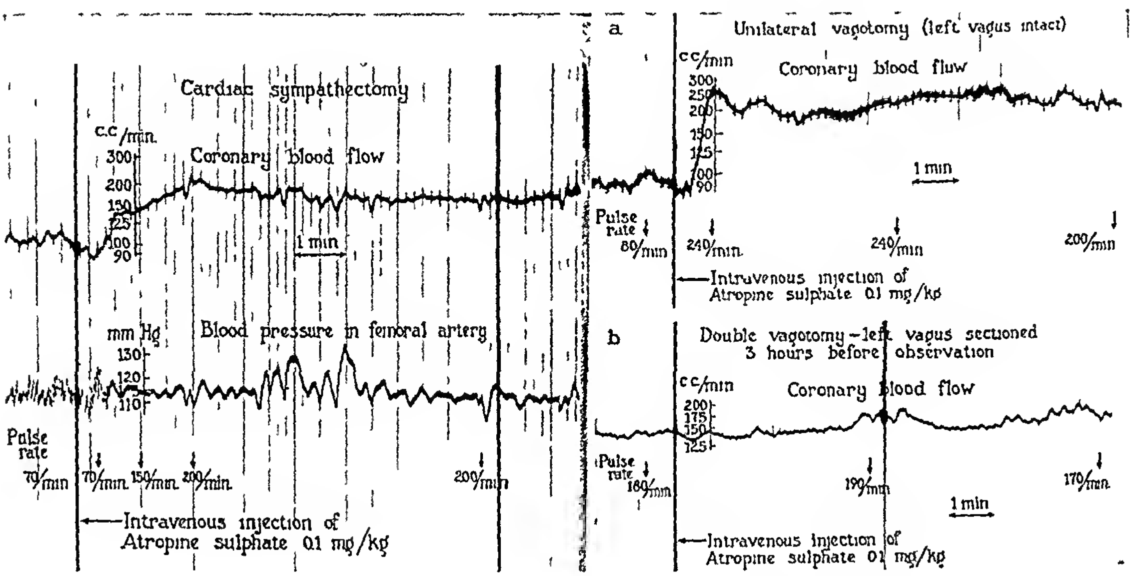

Fig. 1 Fig. 2

Fig. 1. The effect of an intravenous injection of 0.1 mgm. per kgm. of atropine sulfate on the coronary blood flow, pulse rate and blood pressure of a dog after cardiac sympathectomy.

Fig 2. Effect of an intravenous injection of 0.1 mgm. per kgm. of atropine sulfate on the coronary blood flow of a dog *a* before and *b* after the second vagus nerve was sectioned.

flow and heart rate of the dog in this experiment remained above the control values for more than an hour.

The thermostromuhr unit was applied one month and four days after the first sympathetic ganglionectomy. In the absence of the sympathetics but in the presence of the vagi, the heart accelerated to 200 beats each minute, which would indicate that it was under considerable vagal tone, removal of which by the effect of atropine allowed the heart marked acceleration. The acceleration was not the result of sympathetic tone since the sympathetics had been removed (fig. 1).

Effect of atropine after bilateral cervical vagotomy. Whether atropine affects

the coronary blood vessels directly, causing a vasodilatation, was considered and a series of experiments were done in which the vagus nerves were sectioned a month or so previously. In these animals the blood pressure, heart rate and coronary blood flow were unaffected by injections of atropine.

Observations were also made on another series of dogs in which the effect of the drug was observed before and a few hours after section of the remaining vagus nerve. In one experiment the control pulse rate before section of the remaining vagus nerve was 80 beats each minute and the coronary flow was about 100 cc. each minute. Three hours after section of the remaining vagus the control heart rate was about 180 each minute. The coronary blood flow was about 150 cc. each minute. An injection of atropine did not materially alter the control values (fig. 2). The results were the same following double cervical vagotomy whether or not the sympathetic nerves were present.

COMMENT. It appears that the cardiac acceleration resulting from vagal paralysis by injections of atropine sulfate was owing entirely to release of vagal influence or tone. The increased coronary blood flow was not the result of increases of the blood pressure because the blood pressure was not significantly altered. Since the injection of atropine had no effect on the blood pressure, heart rate or coronary blood flow in the totally denervated heart, it may be logically assumed that the increased coronary blood flow resulting from the injection of atropine into animals with innervated hearts and into animals with hearts supplied with only the vagus nerves is not owing to a direct dilator effect of atropine on the coronary vessels. In view of the fact that increases of heart rate alone have been shown to affect coronary flow decisively, one is led to the conviction that the acceleration of the heart, following administration of atropine in sufficient dosage, is responsible for the increased coronary blood flow. The mechanism by which this might be accomplished is, however, unknown.

SUMMARY AND CONCLUSIONS

In the present study the response of the coronary blood flow and heart rate to atropine sulfate has been observed after the following operative procedures: 1, right and left sympathetic ganglionectomy from the eighth or ninth intercostal space anteriorly including the stellate ganglion; 2, double vagotomy in the neck; 3, a combination of procedures 1 and 2. In the absence of the sympathetic nerves, as in procedure 1, atropine caused increases of 25 to 85 per cent in coronary flow and an increase in pulse rate of a similar magnitude. Atropine was without effect on the coronary blood flow, heart rate or blood pressure of vagotomized animals or animals with denervated hearts. It may be concluded that the increased coronary blood flow following injections of atropine is not owing to a direct effect of the drug on the wall of the blood vessel nor is it due to changes of blood pressure. The augmented coronary flow follows the inhibition of vagal tone and is associated with the resulting increased cardiac rate. When all of the evidence is considered it is difficult to escape the conclusion that the increased heart rate itself is responsible for the increased coronary blood

flow following administration of atropine but the mechanism by which it is accomplished is not apparent.

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EFFECTS OF EXERCISE ON THE CORONARY BLOOD FLOW, HEART RATE AND BLOOD PRESSURE OF TRAINED DOGS WITH DENERVATED AND PARTIALLY DENERVATED HEARTS

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In a previous paper (1) we have reported simultaneous observations on the blood pressure, heart rate and coronary blood flow of the dog in response to exercise on a treadmill. It was shown that the changes of heart rate at the beginning of exercise were usually a better criterion of the changes of coronary blood flow than the arterial blood pressure. With the beginning of exercise a rapid increase of coronary blood flow was seen which was accompanied by a greatly accelerated pulse. It has been shown in a relatively large series of experiments that the behavior of the blood pressure is unpredictable during the initial minutes of exercise. It may increase, decrease, be diphasic or remain unchanged. This behavior of the blood pressure supported the conclusion that the increased coronary blood flow observed at the beginning of exercise was not the result of changes of the arterial blood pressure alone.

In another study (2) it was demonstrated that increasing or decreasing the cardiac rate while keeping other factors relatively constant resulted respectively in an increased or decreased coronary blood flow.

The sudden acceleration of the heart in dogs coincident with the beginning of exercise is considered to be owing principally to a reduction of cardiovagal tone (3). It was therefore considered important to study the effects of exercise on the heart rate, blood pressure and coronary blood flow of dogs after denervation and partial denervation of the heart.

METHODS. Denervation of the heart was done in stages. Right vagotomy was usually done first. Through an incision low in the neck and over the anterior aspect of the thorax the vagus nerve was exposed. This nerve and the brachial artery were mobilized in such a manner as to make possible the identification and section of all the branches of the vagus except the recurrent nerve, which was preserved. At the second stage sympathetic ganglionectomy was done on the left side from the eighth costal interspace anteriorly including the

stellate ganglion. The third stage consisted of the same operation on the sympathetic ganglia of the right side. In the fourth stage the left vagus nerve was sectioned about midway in the neck. When it was desired only to vagotomize the heart the two operations were done as just described in stages one and four.

The coronary blood flow was measured by the thermostromuhr method. In all experiments the unit was applied to the circumflex branch of the left coronary artery before or after the last stage of cardiac denervation; that is, before or after sectioning the remaining vagus nerve. By applying the unit before sectioning the remaining vagus nerve, control data on blood flow, blood pressure and heart rate obtained on a partially denervated heart could be compared with those from a totally denervated heart. When data on animals on which sympathetic ganglionectomy alone had been performed were desired both operations were done before the thermostromuhr unit was applied to the coronary vessel.

In the experiments on animals that had vagotomized hearts both nerves were sectioned in some cases while in other cases only the right vagus was sectioned before the application of the thermostromuhr unit. Thus in the latter experiments the effects of the loss of one vagus could be compared with the results following total vagotomy.

All of the major operative procedures were done with the animals under general anesthesia and sterile technic was observed. The blood pressure was recorded optically from a cannulated femoral or carotid artery using a glass spoon manometer (4). The heart rate was recorded by an electrocardiograph. The dogs were trained to exercise on the treadmill before and during their preparation for the various experiments.

Except in cases of persistent vomiting such as occurred in the vagotomized animals, when repeated feeding was necessary, the dogs were fed each day after the observations had been completed. Food was withheld at other times.

Routinely the animals were exercised for a total of fifteen minutes: five minutes with the treadmill running horizontally, five minutes with it elevated at about an angle of 15 degrees and five minutes again in the horizontal position.

Results. *Cardiac sympathectomy.* A series of experiments was done on animals 24 to 124 days following the first operation in which sympathetic ganglionectomy was done. The question of whether regeneration of the sympathetic nerves had occurred could not justifiably be raised concerning experiments that had been done within twenty-four days after the first stage of cardiac sympathectomy. The results of all such experiments were not essentially different from those obtained previously on dogs that had intact nervous systems. Coronary blood flow was markedly increased with increments of the rate of work. As in experiments reported previously (1) increases of the coronary blood flow sometimes occurred in the presence of a decreased blood pressure. Increases of coronary blood flow were always associated with an increased heart rate. This has been taken as indicating that factors associated with heart rate alone are capable of profoundly influencing coronary blood flow in a manner that is thus far unexplained (table 1).

Bilateral cervical vagotomy. In some experiments both vagus nerves were

TABLE 1

Effect of exercise on heart rate, coronary blood flow and blood pressure of dogs after cardiac sympathectomy

DOG		CONTROL	1 MINUTE AFTER TREADMILL STARTED	1 MINUTE AFTER TREADMILL ELEVATED	1 MINUTE AFTER TREADMILL LOWERED	1 MINUTE AFTER TREADMILL STOPPED	5 MINUTES AFTER TREADMILL STOPPED	10 MINUTES AFTER TREADMILL STOPPED	REMARKS
1	Heart rate Blood flow*	90 70	120 115	160 132	140 115	130 89	 85	130 82	6-5-40: Left cardiac sympathectomy 8-7-40: Right cardiac sympathectomy 10-9-40: Unit applied 10-14-40: Flow was 115 cc. per minute, 10 seconds after treadmill was elevated
	Blood flow Blood pressure	69 101	90 97	145 111	120 132	105 121	82		10-15-40: Left femoral artery cannulated
2	Blood flow Blood pressure	70 108	137 102	162 119	155 127	120 111	108 110		12-18-40: Left cardiac sympathectomy 1-8-41: Right cardiac sympathectomy 2-26-41: Unit applied 3-6-41: Left femoral artery cannulated
3	Heart rate Blood flow	130 118	185 285	190 215	190 205	160 170	150 118		1-18-40: Left cardiac sympathectomy 1-8-41: Right cardiac sympathectomy 2-6-41: Unit applied 2-8-41
	Heart rate Blood flow	110 65	160 170	190 215	170 200	130 105	120 76		2-10-41
	Heart rate Blood flow Blood pressure	130 98 130	180 155 160	210 190 162	220 190 162	180 160 ?	175 170 170	140 89 151	2-12-41: Left femoral artery cannulated

* Blood flow = cc. per minute.

sectioned as long as thirty days before the application of the thermostromuhr unit. These animals had fully recovered from the effects of the operation itself but continued to vomit several times a day as long as they lived. In spite of the persistent vomiting the animals, when fed frequently, retained enough food to maintain the body weight near the control value.

In agreement with the findings of Samaan (3) the heart rate increased to about 180 beats each minute immediately after sectioning the second vagus nerve which was usually done under infiltration anesthesia with procaine hydrochloride. When the remaining vagus nerve was sectioned an increase of coronary blood flow always occurred. In one experiment a marked transient elevation of blood pressure was observed following section of the second vagus nerve. As a rule the increased heart rate remained maximal for only a few minutes after the second vagus was cut. The heart rate gradually decreased and twenty to thirty minutes after total vagotomy it had usually stabilized at about 140 to 150 beats each minute. The resting heart rate did not in every case remain indefinitely at about 140 beats each minute but continued in some animals to decrease gradually over a period of several weeks until it had reached 120 to 90 beats each minute. That this decreased rate was not owing to a restoration of vagal influence was demonstrated by the inability of intravenously administered atropine sulfate, in doses of 0.1 to 0.2 mgm. for each kilogram of body weight to affect the heart rate. In some animals the heart rate and coronary blood flow slowly decreased over a period of several days following section of the second vagus nerve, but the values obtained before sectioning the second vagus were not usually reached.

Simultaneously with the beginning of exercise the heart with one or both vagi invariably acclerated greatly, frequently doubling its control rate within thirty seconds. The coronary blood flow at the same time increased rapidly, sometimes showing increases of nearly 100 per cent. The initial responses of the blood pressure were unpredictable. After total vagotomy the heart lost its power of marked acceleration at the beginning of exercise or with increasing rate of work. Ten to twenty beats each minute was usually the limit of acceleration in either case. In general in the absence of an increased heart rate but in the presence of a much elevated blood pressure the coronary flow showed large increases. On the other hand, in the presence of a slightly decreased or a constant blood pressure and in the absence of cardiac acceleration the coronary flow remained practically unchanged. After vagotomy the coronary blood flow at the beginning of exercise was apparently influenced more by the arterial blood pressure than by any other single factor (table 2).

Total cardiac denervation. Observations were made on animals whose hearts had been totally denervated, the operative procedures for which have already been described. By applying the thermostromuhr unit to the coronary artery after cardiac sympathectomy and unilateral vagotomy or previous to sectioning the remaining vagus nerve, control data on the totally denervated hearts were obtained.

It has been shown in another section of this paper that the response of the blood pressure, heart rate and coronary blood flow to exercise in animals that had

TABLE 2

Effect of exercise on heart rate, coronary blood flow and blood pressure of vagotomized dogs

DOG		CONTROL	1 MINUTE AFTER TREADMILL STARTED	1 MINUTE AFTER TREADMILL ELEVATED	1 MINUTE AFTER TREADMILL LOWERED	1 MINUTE AFTER TREADMILL STOPPED	5 MINUTES AFTER TREADMILL STOPPED	10 MINUTES AFTER TREADMILL STOPPED	REMARKS
4	Heart rate	110	130	150	130	120		120	3-27-40: Sectioned left vagus 4-17-40: Sectioned right vagus 5-15-40: Unit applied 5-20-40
	Blood flow*	48	90	145	82	63		64	
	Heart rate	140	140	150	140	140		140	5-21-40: Number 1 left femoral artery cannulated
	Blood flow	62	122	136	122	80		65	
	Blood pressure	100	140	145	135	112		100	
	Heart rate	110	130	140		130	130		5-21-40: Number 2
	Blood flow	62	96	136	122	62			
	Blood pressure	85	100	118	106	90			
5	Heart rate	70	130	180	150	110	125		6-18-40: Sectioned right vagus 11-20-40: Unit applied 11-26-40: Control
	Blood flow	98	218	390	180	113	113		
	Heart rate	180	190	220	190	180	180		11-27-40: After sectioning left cervical vagus
	Blood flow	163	280	?	215	180	155		
	Heart rate	170	170	180	170	160	170		11-28-40
	Blood flow	120	165	235	125	108	105		
	Heart rate	150	160	180	160	150	150	150	11-29-40
	Blood flow	125	275	?	125	110	106		
	Heart rate	160	170	175	170	150	160		12-2-40: Left femoral artery cannulated
	Blood flow	145	192	395	210	96	102		
	Blood pressure	93	121	153	153	114	110		

* Blood flow = cc. per minute.

sympathectomized hearts was the same as seen in those that had intact sympathetic nervous sytems. The evidence secured in the present series of experi-

ments is confirmatory of this finding. The results of observations made on animals on which bilateral sympathectomy and unilateral vagotomy had been performed were indistinguishable from the results obtained on animals that had intact nervous sytems (upper tracing in fig. 1). When the remaining vagus was sectioned a profound difference in the results during exercise was seen (fig. 1). In brief, the results were strikingly like those described in experiments in which only bilateral cervical vagotomy had been done.

In one experiment the blood pressure was recorded before, during and after section of the remaining vagus nerve. In a control observation the blood pressure decreased slightly when exercise was begun while the coronary blood flow definitely increased but the heart rate accelerated only 20 beats each minute. The typical response of blood pressure, heart rate and coronary flow followed an augmentation of the rate of work. After recovery from the effects of this period of exercise the remaining vagus was isolated and prepared for sectioning. Following a control period of five minutes of exercise the remaining vagus was cut. The blood pressure rose immediately from 125 to 170 mm. of mercury. The heart rate increased 40 beats each minute and the coronary blood flow increased about 100 per cent. The increased heart rate remained maximal for only a few minutes, having decreased 10 beats each minute five minutes following section of the vagus. After an interval of five minutes the treadmill was again started in the horizontal position. The effect on the blood pressure was practically negative. The heart rate increased 10 beats each minute. The coronary blood flow was practically unaffected by the exercise. Five minutes later the rate of work was augmented by elevating the treadmill. The immediate effect on the blood pressure was a decrease which was reflected in the coronary blood flow. After a minute the blood pressure and coronary blood flow had increased above the control and the heart rate had accelerated to 190 beats each minute. A few days later the heart rate had decreased to 90 beats each minute when the animal was at rest (dog 8 in table 3).

Rectal temperature and exercise. Since changes of body temperature influence the rate of the heart it seemed important to determine the effect on temperature that resulted from the standard rate of exercise. The rectal temperature was taken before exercise and one minute after the treadmill was stopped at the completion of the usual exercise of fifteen minutes' duration. In nine experiments on sympathectomized dogs the increase of temperature was from 1 to 1.8°F. The average control temperature was 102.5°F., while the average at the completion of exercise was 103.6°F. In six observations on animals all of whose cardiac nerves except the left vagus had been removed the increase of temperature after exercising for fifteen minutes varied from 0.6 to 2.8°F. The average control temperature was 101.6°F. and the average temperature at completion of exercise was 103.1°F. Seven observations on vagotomized dogs showed increases from 0.4 to 2.8°F. resulting from periods of exercise of fifteen minutes' duration. Increases of temperature as great as just indicated undoubtedly cause an acceleration of the heart. It is doubtful whether the increased temperature accompanying the first 90 to 120 seconds of exercise was sufficient to

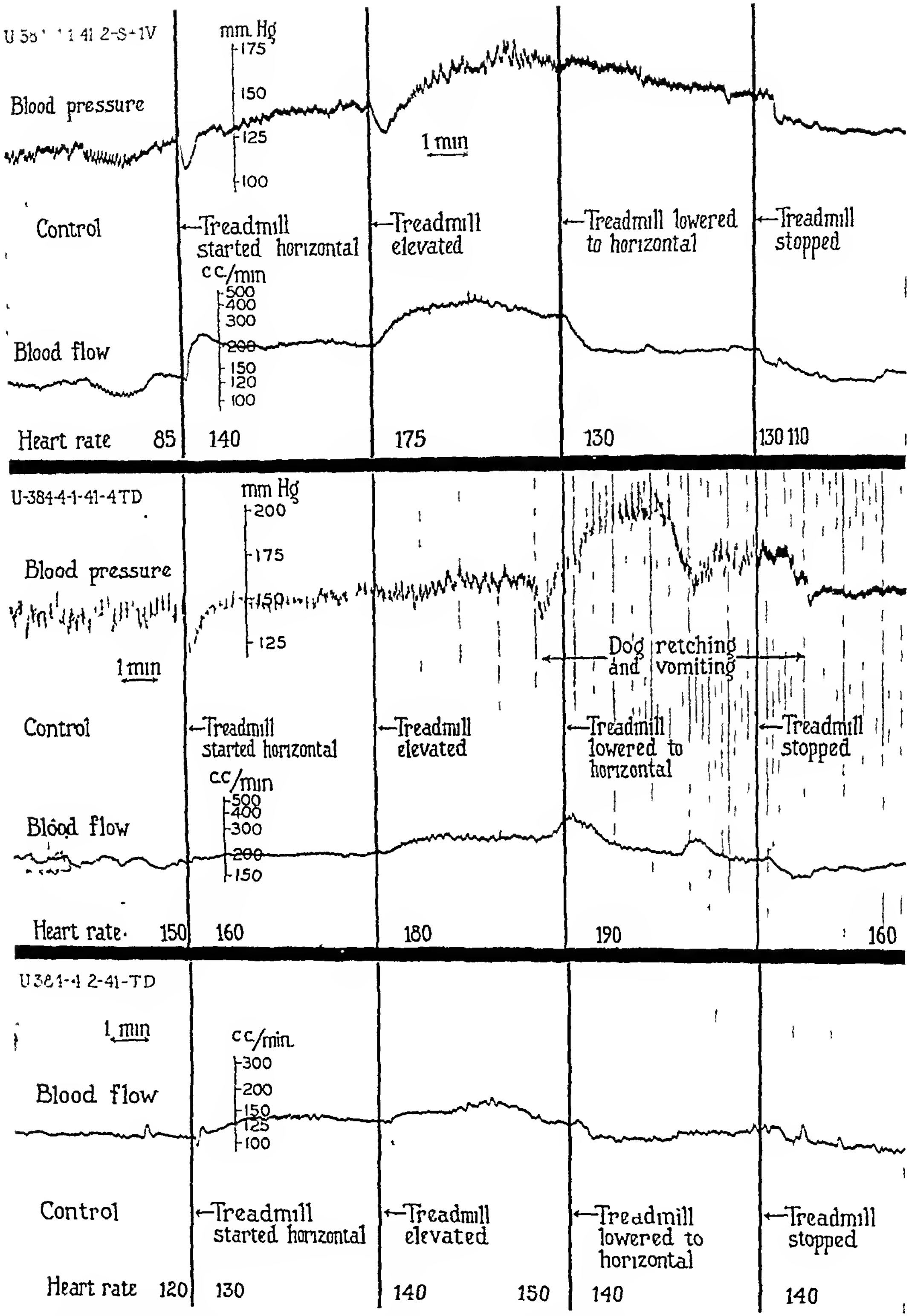

Fig 1. The response of the blood pressure, coronary blood flow and heart rate of a dog after, first, partial and then total denervation of the heart. In the upper tracing the left vagus nerve is still intact. The middle tracing was taken thirty-five minutes after sectioning the left vagus nerve. The bottom record of coronary blood flow was made about twenty-four hours later.

TABLE 3

Effect of exercise on heart rate, coronary blood flow and blood pressure having partially and totally denervated hearts

DOG		CONTROL	1 MINUTE AFTER TREADMILL STARTED	1 MINUTE AFTER TREADMILL ELEVATED	1 MINUTE AFTER TREADMILL LOWERED	1 MINUTE AFTER TREADMILL STOPPED	5 MINUTES AFTER TREADMILL STOPPED	10 MINUTES AFTER TREADMILL STOPPED	REMARKS
6	Heart rate Blood flow*	90 29	130 53	170 90	140 94	110 80		100 66	8-28-40: Right cervical vagotomy 9-11-40: Left cardiac sympathectomy 10-16-40: Right cardiac sympathectomy 11-6-40: Unit applied 11-13-40: Heart denervated except left vagus
	Heart rate Blood flow	130 42	150 57	200 105	200 67	170 55	 51		11-15-40: Left vagus sectioned, total cardiac denervation
	Heart rate Blood flow	130 34	150 45	185 70	160 49	140 46	 36		11-16-40
7	Heart rate Blood flow	105 150	140 190	180 ?	155 206	130 175	110 171		1-16-41: Right cervical vagotomy 1-29-41: Left cardiac sympathectomy 2-13-41: Right cardiac sympathectomy 3-19-41: Unit applied 3-25-41: Heart denervated except for left vagus
	Heart rate Blood flow	100 143	130 165	170 290	160 165	140 142	120 146		3-26-41: Maximal flow with treadmill elevated 340 cc. per minute
	Heart rate Blood flow	90 165	130 178	165 285	150 185	120 132	110 150		3-27-41
	Heart rate Blood flow	75 103	 180	 220	 144	100 115	100 118		3-31-41: Number 1

TABLE 3—(*Concluded*)

DOG		CONTROL	1 MINUTE AFTER TREADMILL STARTED	1 MINUTE AFTER TREADMILL ELEVATED	1 MINUTE AFTER TREADMILL LOWERED	1 MINUTE AFTER TREADMILL STOPPED	5 MINUTES AFTER TREADMILL STOPPED	10 MINUTES AFTER TREADMILL STOPPED	REMARKS
	Heart rate	75	130	170	140	115	100		3-31-41: Number 2
	Blood flow	109	185	275	175	128	143		
	Heart rate	70	130	160	120	110	90		4-1-41: Number 1
	Blood flow	112	165	235	150	113	112		
	Heart rate	85	140	175	130	110	100		4-1-41: Number 2 Left carotid cannulated
	Blood flow	137	215	340	190	150	137		
	Blood pressure	121	129	140	160	128	120		
	Heart rate	150	160	180	190	160	160		4-1-41: Twenty-five minutes after sectioning left vagus
	Blood flow	185	205	260	275	165	210		
	Blood pressure	141	147	151	193	159	151		
	Heart rate	120	130	140	140	140	130		4-2-41
	Blood flow	108	127	150	113	108	100		
									1-16-41: Right cervical vagotomy 1-29-41: Left cardiac sympathectomy 2-13-41: Right cardiac sympathectomy 3-19-41: Unit applied
8	Heart rate	130	140	190	170	140	130		3-25-41
	Blood flow	172	210	300	260	185	171		
	Heart rate	110	130	180	170	140	140		3-26-41
	Blood flow	150	165	250	225	174	165		
	Heart rate	130	150	180	170	140	130		3-27-41
	Blood flow	160	200	256	215	185	185		
	Heart rate	100	120	170	160	130	120	110	3-28-41
	Blood flow	165	225	330	295	280	247	230	
	Blood pressure	121	113	135	149	143	139	141	Left carotid artery cannulated 3-28-41: Left vagus sectioned
	Heart rate	90	100	140	120	110	100		3-31-41
	Blood flow	68	76	120	111	96	76		
	Heart rate	100	120	140	130	110	110		4-1-41
	Blood flow	104	104	130	122	120	110		
	Heart rate	95	100	130	120	110	100		4-2-41
	Blood flow	100	110	130	125	105	95		

* Blood flow = cc. per minute.

affect the heart rate or coronary blood flow materially but the elevated temperature was undoubtedly one of the factors responsible for the augmented pulse rate resulting from increments of the rate of work.

COMMENT. It must not be overlooked that the basal or resting heart rate and coronary blood flow of the recently vagotomized and totally denervated hearts were sometimes nearly twice as great as before denervation. It appeared that these hearts were being supplied with more blood than was required for their basal needs. When exercise was begun there was sufficient flow of blood to meet the needs of the heart in some cases for as long as five minutes. With further increments of the rate of work additional coronary blood flow resulted from increases of arterial blood pressure or from the operation of other mechanisms. The influence of increases of body temperature, the accumulation of metabolites and the rôle of hormones must be considered as possible factors in the production of the increased coronary blood flow that resulted from high rates of work continued for several minutes. It is doubtful whether these last-named factors played an important part in the increased coronary flow sometimes seen during the first ten to thirty seconds of exercise.

The nervous control of the coronary blood vessels has been under discussion for many years but complete agreement has not been reached on any particular phase of the problem. This is owing in part at least to the difficulty of devising and executing crucial experiments as well as repeating in all respects the work of others, and also in part to lack of complete confidence in any method yet developed for measuring coronary blood flow. In the present state of the science of physiology most methods at best probably give only a very rough approximation of what is taking place in the undisturbed organ.

With all the limitations acknowledged with respect to the present experiments, which have been in progress for a number of years, we submit that sufficient data are presented in this report to indicate rather conclusively the dominant rôle of the vagus in control of cardiac circulation. In not a single instance has there been any evidence that the sympathetic nerves exercised a tonic action on the heart or coronary vessels. A sympathectomized heart was indistinguishable from a normal heart as to rate and coronary blood flow. In every series of experiments the tonic action of the vagus was strongly evident. A dramatic change of rate or coronary flow was not seen except when the heart was deprived of both vagus nerves. So long as one vagus supplied the heart its reaction to exercise as respects rate and coronary flow was indistinguishable from that of the fully innervated heart. In the absence of the cardiac sympathetic nerves the sectioning of the remaining vagus nerve was followed by marked accleration of heart rate and augmentation of coronary blood flow. The response of the rate and coronary blood flow of the totally denervated heart to moderate exercise was in many instances completely negative whereas the fully innervated heart showed marked acceleration and increased coronary blood flow with the same rate of exercise. The results of our experiments on the trained dog strongly support the findings of Anrep and Segall (5) on the innervated and denervated heart-lung preparation as regards the tonic vagal control of coronary blood flow and like-

wise our results support the findings of Samaan (3) with regard to the influence of the vagus on the heart rate of exercising dogs.

SUMMARY AND CONCLUSIONS

Observations have been made on the coronary blood flow, heart rate and blood pressure of trained dogs after the following procedures: 1, bilateral sympathetic ganglionectomy, from the eighth costal interspace anteriorly including the stellate ganglion; 2, double cervical vagotomy; 3, right vagotomy followed by left vagotomy; 4, cardiac sympathectomy and right cervical vagotomy, followed by left cervical vagotomy. Blood flow in the circumflex branch of the left coronary artery was observed by use of the thermostromuhr. Blood pressure was recorded optically from a cannulated femoral or carotid artery. The heart rate was observed electrocardiographically.

The effects of exercise on animals that had sympathectomized hearts were not essentially different from results obtained in animals that had innervated hearts. In both series exercise produced increased coronary blood flow, pulse rate and blood pressure. The observations were made 24 to 124 days after sympathetic ganglionectomy. The effects of exercise were very similar in animals on which complete cardiac denervation had been performed and those lacking only the vagi. Loss of the vagi affected cardiac acceleration profoundly. Vagotomized hearts increased only about 10 to 20 beats each minute with increments in the rate of work. This was true whether or not the sympathetic nerves were present. In the absence of marked acceleration and elevation of blood pressure the coronary blood flow was not affected by exercise. In animals that had vagotomized or totally denervated hearts the coronary blood flow appeared to be influenced chiefly by the blood pressure.

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THE DISAPPEARANCE OF T-1824 AND STRUCTURALLY RELATED DYES FROM THE BLOOD STREAM

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The rate at which vital dyes escape from the blood stream has for several years been of practical interest in connection with the determination of plasma volume with the dye method. Although observations have been made on a large variety of dyes (Dawson, Evans and Whipple, 1920; and others), little has been accomplished to explain the remarkable ability of certain dyes to leave the blood slowly (notably T-1824 and brilliant vital red) or to account for the striking differences in rate of disappearance exhibited by dyes of similar structure and molecular weight. These questions have an important bearing on the interpretation of experiments in which dyes are employed for measuring the plasma volume or for demonstrating local or overall changes in the permeability of the vascular bed. The present investigation concerns the behavior of four dyes—T-1824, trypan blue, niagara sky blue and niagara sky blue 6B—and the interpretation of their behavior in the light of recent electrophoretic studies (Rawson, 1943).

Experimental procedure. Commercial batches of trypan blue have long been known to contain considerable amounts of a red component which has been found to be more diffusible than trypan blue itself (von Möllendorf, 1914). Impure samples would therefore be unsuitable for the present studies and give an erroneous impression of the rate at which the dye leaves the blood stream. For these studies the dye was purified[1] until the "capillary test" showed that all traces of the red impurities had been removed. By the same token the other three dyes were known to be free of contaminating isomers.

Four adult mongrel dogs, one male and three females, accustomed to the procedure of blood volume determinations, were selected for the tests. They were given water *ad libitum* but no food for twenty hours before each experiment. The tests were carried out with the dog resting quietly on an animal board. After withdrawal of a dye-free sample of blood from one of the jugular veins, a measured amount of the dye to be tested was injected into this vein with a calibrated syringe. Samples (2 cc.) were then collected without stasis from the opposite jugular vein at exactly 5, 10, 15, 20, 30, 40, 50, 65, 80 and 95 minutes after the injection. The dye determinations were made on the serum with a Koenig-Martens visual spectrophotometer, each dye being read at the wave

[1] The dyestuff is precipitated 4–5 times with sodium acetate to remove the alcohol insoluble salts. The final precipitate is then dissolved in a small volume of hot water and immediately poured into several volumes of alcohol. The dye forms a fine precipitate which settles out, whereas the sodium acetate and "red component" remain in solution. The alcohol precipitation is repeated until the "capillary test" is negative for red.

length where its spectral absorption in serum is maximal (Gregersen and Gibson, 1937). For injection the dyes were made up in 0.5 per cent solution in distilled water. These solutions were standardized by determining the optical density at a dilution of 1:250 in serum. The values are given in table 1.

RESULTS. In order to assemble the dye curves for direct comparison and to calculate in each instance the fraction of dye which has escaped or been removed from the plasma over a given period after the injection it becomes necessary to express the plasma dye concentrations in terms of an initial concentration. We shall use the term *initial concentration* here to mean the concentration that obtains if 100 per cent of the dye is uniformly mixed with the circulating plasma. This value is obviously not directly measurable because of the inescapable fact that by the time the dye is uniformly distributed some of it has already left the bloodstream. The time required for mixing and the amount of dye lost during this period must therefore be considered in estimating the initial concentration.

TABLE 1

DYE	WAVE LENGTH	OPTICAL DENSITY 0.5%—1:250 IN SERUM
	$m\mu$	
T-1824*	620	1.65
Niagara sky blue 6B	620	1.34
Trypan blue	605	1.41
Niagara sky blue	600	0.95

* Supplied by Warner Institute of Therapeutic Research.

From a consideration of the speed of the circulation of the blood it has been generally inferred that mixing would be effected in from 2 to 5 minutes (Erlanger, 1921). This conclusion is apparently supported by the fact that within 2 to 3 minutes after the injection of dye there is no longer a measurable difference in dye concentration among samples taken simultaneously from various regions of the body (Gilder, Müller and Phillips, 1940). It is to be noted, however, that this test does not prove that the dye has been mixed with all of the plasma in the vascular bed.

Time-concentration curves have been variously interpreted. Keith, Rowntree and Geraghty (1915) who introduced the dye method considered that mixing was complete in 4 to 6 minutes. Subsequent studies with more precise methods of measuring dye concentration have indicated that the mixing time may be considerably longer (Gregersen et al., 1935–39; Gibson and Evans, 1937; Price and Longmire, 1942). Robinow and Hamilton (1940) lean in the opposite direction, stating that "the shape of the disappearance curve suggests . . . that mixing of the dye is complete after two or three complete circulations." Hahn and his associates (1942) find that the tagged erythrocytes mix completely in 2 to 4 minutes with all the red cells and with about four-fifths of the plasma. Hahn puts forth the proposition that the remaining one-fifth of the plasma is present largely as a stagnant or slowly moving peripheral film lining the walls of the small vessels and capillaries and that dye reaches this film slowly by diffusion from the axial stream. His estimate of the amount of plasma which is slowly mixed seems rather high. In the first place the change in dye concentration (T-1824) during what Hahn terms the "second phase" of mixing is seldom greater than 5 to 10 per cent. In the second place it is probable that only about 10 per cent of all the blood is present in the capillary bed (Bazett, 1941) and of this a considerable fraction must be in active circulation. The diffusion of dye from the axial stream into the

peripheral films may possibly be a factor during the second phase of mixing, but the phenomenon of "vasomotion" (Chambers, 1942) and the intermittent capillary flow (Fulton and Lutz, 1940) would seem to be more important.

TABLE 2

DOG NO.	SEX	B.WT.	DYE	LINEAR PLOT (OPTICAL DENSITY VS. TIME)					LOG PLOT (LOG OF OPTICAL DENSITY VS. TIME)		SQUARE ROOT PLOT (OPTICAL DENSITY VS. $\sqrt{T}$)	
				Optical density: 0 min. (by extrapolation)	Optical density: 60 min.	Dye lost in 60 min.	Plasma volume		Opt. dens. 0 min. (by extrapolation)	Plasma volume	Opt. dens. 0 min. (by extrapolation)	Plasma volume
Date: 7-9-41. T-1824												
		kgm.	cc.			per cent	cc.	cc. kgm.		cc.		cc.
1	F	13.90	4.06	1.37	1.28	6.5	568	40.8	1.37	568	1.40	556
2	M	11.77	4.07	1.24	1.11	10.4	631	53.7	1.25	621	1.305	605
3	F	10.84	3.08	0.915	0.82	10.3	637	58.8	0.915	637	0.975	598
4	F	10.20	3.07	1.01	0.935	7.5	584	57.7	1.01	584	1.12	526
Averages...		11.68				8.8	605	52.7		603		571
Date: 6-19-41. Niagara Sky Blue 6B (D-1824)												
1	F	13.35	5.03	1.53	1.35	11.7	512	38.4	1.535	510	1.58	495
2	M	12.02	5.05	1.30	1.06	18.4	604	50.2	1.30	604	1.365	575
3	F	11.37	5.05	1.14	0.98	14.0	688	60.5	1.14	688	1.19	659
4	F	10.40	4.06	1.105	0.93	15.8	573	56.1	1.105	573	1.11	570
Averages...		11.79				15.0	594	51.3		594		575
Date: 6-26-41. Trypan Blue (T-1836)												
1	F	13.51	5.03	1.44	0.86	40.3	582	43.0	1.48	567	1.51	555
2	M	12.34	5.05	2.42	1.35	44.2	736	59.6	2.45	727	2.54	701
3	F	10.71	5.05	1.16	0.67	42.2	711	66.4	1.18	699	1.25	660
4	F	10.07	4.06	1.04	0.68	34.6	640	63.5	1.05	634	1.11	600
Averages...		11.66				40.3	667	58.1		657		629
Date: 6-14-41. Niagara Sky Blue (D-1836)												
1	F	13.06	5.03	2.20	1.37	37.7*	601	46.1	2.2	601	2.345	563
2	M	12.28	5.05	2.00	0.93	53.5	663	54.0	2.0	663	2.2	603
3	F	11.24	5.05	1.90	0.85	55.3	698	62.0	1.9	698	1.96	676
4	F	10.19	4.06	1.80	0.85	52.7	594	58.3	1.82	587	1.82	587
Averages...		11.69				53.8	639	55.0		637		607

* Not included in average.

After a dye is uniformly mixed with the circulating plasma and until it re-enters the circulation by way of the lymph, the time-concentration curve presumably defines the rate at which the dye is passing out of the plasma compartment. From lymph studies with brilliant vital red (Smith, 1925) and with T-1824 (Ferrebee, Leigh and Berliner, 1941) it

may be concluded that re-entry of dye does not modify the disappearance curve perceptibly for at least an hour after the injection. The amount of dye returned to the blood stream through the thoracic duct during this time is only about 2 per cent of the total dye injected. Hence if one accepts the proposition that the curve from 10 minutes up to about one hour represents a continuous process which begins at the moment of injection, then the initial concentration should be obtainable by appropriate extrapolation of this curve to the zero time ordinate (time of injection). The values for the initial concentration (optical density at 0 min.) given in table 2 under the heading "Linear Plot" were obtained by simply extrapolating a smooth curve drawn through the points from 10 to 65 minutes. Other methods of making the extrapolation will be considered presently.

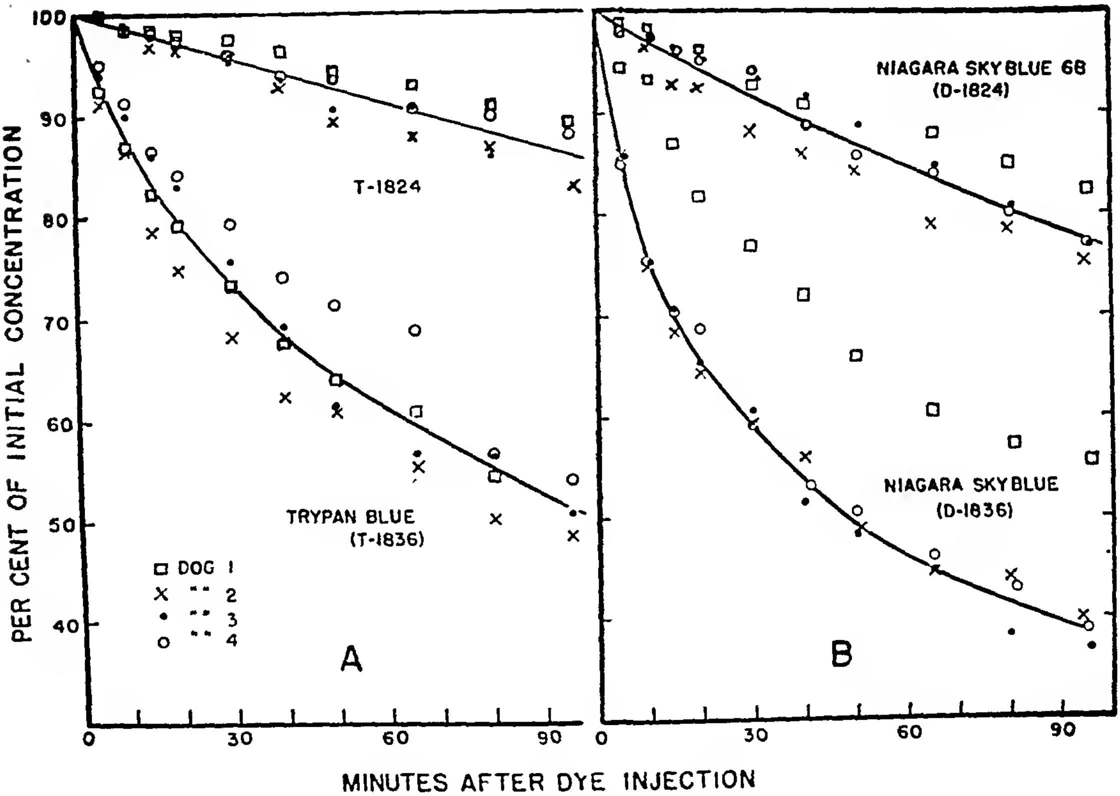

Fig. 1. Showing the rates of disappearance from the bloodstream of four structurally similar dyes. Each curve represents the average of four tests, except in the case of niagara sky blue where the results on dog 1 were excluded in calculating the average.

The graphs obtained by plotting the optical density determinations as per cent of the extrapolated initial value are shown in figure 1. It will be seen that with one exception, the test with niagara sky blue on dog 1, the results from dog to dog are quite consistent, showing clearly the differences in the escaping tendencies of the dyes. The heavy line drawn through the group of determinations with each dye has been plotted from the calculated averages of per cent dye remaining at various times after injection. Table 2 shows the per cent dye lost in 60 minutes in each test and the average loss for each dye. The value 8.8 per cent for T-1824 agrees closely with the average of several hundred determinations made on dogs in this laboratory during the past few years.

The time-concentration curve of each of the dyes under consideration here presumably follows some mathematical function which, if known, would greatly facilitate reliable extrapolation and derivation of the initial concentration. On a linear plot the disappearance curve of T-1824 up to one or two hours is often indistinguishable from a straight line (see fig. 1), and for the practical purpose of extrapolation to calculate the plasma volume it may be considered as such. That it is not actually a linear function can be demonstrated by exaggerating the vertical scale (concentration) or by compressing the horizontal scale (time). The curve then takes on the character of a logarithmic function, and indeed if the data are plotted as the logarithm of concentration against time the result is,

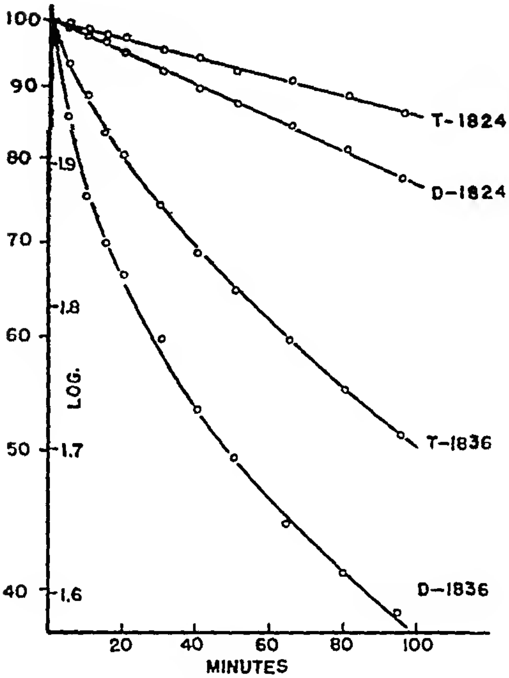

Fig. 2

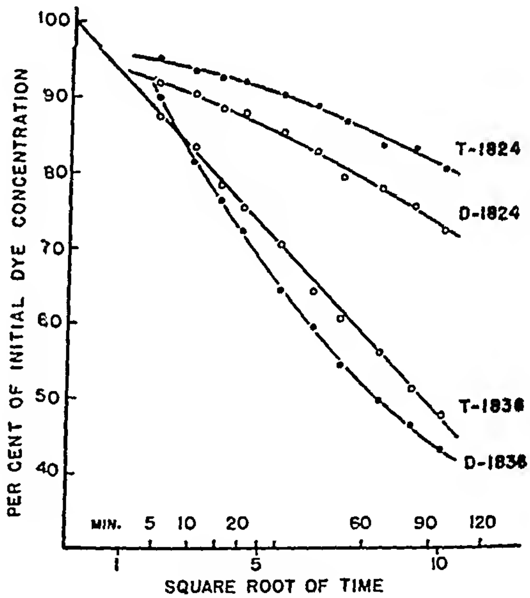

Fig. 3

Fig. 2. Semilog plot of the average values used in constructing the curves shown in figure 1.

Fig. 3. The same data with dye concentration plotted against the square root of time.

as far as one can tell, a straight line for a period of at least an hour after the injection (see fig. 2). Extrapolation gives initial values for T-1824 which are essentially identical with those obtained by extrapolation of the linear plot (see table 2). With niagara sky blue 6B, the semilog plot also gives a straight line and the extrapolated values agree with those from the linear plot. The method is however of little help in dealing with the data on trypan blue and niagara sky blue, for as may be seen in figure 2 the derivation of the initial concentration still involves the extrapolation of a curve.

The same data are shown in figure 3 plotted against the square root of time[2]

[2] The data were submitted to Dr. B. G. King and the values in table 2 are based on his decision as to where the line should be drawn through the points.

(King, Oppenheimer and Cole, 1943). As would be expected, the extrapolation of the curves gives higher values for the initial concentration than on the linear or semilog plot (see table 2).

A comparison of the plasma volumes determined with the four dyes (table 2) reveals differences which do not represent actual changes in volume. The determinations with T-1824 and niagara sky blue 6B give essentially the same average values for the group (605 and 594 cc.), whereas those obtained with trypan blue and niagara sky blue are notably higher (667 and 639 cc.). The discrepancy between the plasma volumes as determined with trypan blue and T-1824 was subsequently confirmed by running both tests on the same dog within a period of three hours. In two such experiments on dogs 1 and 4 the plasma volumes with trypan blue were respectively 568 and 735 cc. The values obtained 2 to 3 hours later with T-1824 were 506 and 667 cc. respectively. (The per cent trypan blue lost during the first hour after injection was 43.2 in dog 1 and 40.9 in dog 4.) The reasons for these discrepancies will be considered in the following discussion.

Discussion. If the observations presented above are examined in relation to the chemical structure of the four dyes it becomes evident that we are dealing here with two modifications in structure which independently influence the escaping tendency of the dyestuff. It will be noted that trypan blue and T-1824 are tolidine dyes, whereas niagara sky blue and niagara sky blue 6B are dianisidine dyes. The only other structural difference among the dyes is in the position of the sulphonic acid radicals on the naphthalene rings. The structural relationships are at once apparent if one adopts a descriptive terminology to conform with the name T-1824. Thus trypan blue may be designated as T-1836, niagara sky blue as D-1836, and niagara sky blue 6B as D-1824.

The results included in figure 1 and table 2 show that a shift in the sulphonic acid radicals from the 2–4 to the 3–6 positions profoundly alters the disappearance rate. In the case of the two tolidine dyes this modification in structure increases the fraction of dye lost in the first hour from an average of about 9 per cent to 40 per cent (cf. T-1824 and T-1836, fig. 1A). In the case of the dianisidine dyes (cf. D-1824 and D-1836, fig. 1B), the same change in structure raises the fraction lost from 15 per cent to 54 per cent. Furthermore, a change from the tolidine to the dianisidine structure also increases the disappearance rate. With the 1824 form of the naphthalene ring this change increases the loss of dye in the first hour from 8 per cent to 15 (cf. T-1824 and D-1824, fig. 1), and with the 1836 naphthalene ring it raises the loss from 40 per cent to 54 (cf. T-1836 and D-1836, fig. 1).

What is the explanation of these differences in the escaping tendency of dyes so closely related structurally? A number of facts concerning the behavior of well-known vital dyes lead one to suspect that they combine with the plasma proteins. There is a good deal of indirect evidence that this is true, for example, of T-1824. This dye leaves the blood stream at approximately the same rate as antibody globulin (Culbertson, 1934). Furthermore, at plasma levels required for the determination of plasma volume T-1824 is not found in the urine unless the latter also contains protein. It is also known that protein stabilizes

the spectral absorption curves of T-1824 and increases the solubility of the dye in sodium chloride (Gregersen and Gibson, 1937). The fact that T-1824 is not removed from plasma by precipitating the fibrinogen with heat and that all the dye in whole blood is recovered in the serum after normal clotting has taken place (Gregersen and Schiro, 1938) would appear to exclude fibrinogen from any part in the binding of the dye in the blood stream.

Direct evidence for the binding of various dyes by protein has been obtained by Rawson (1943) with the electrophoretic technique of Tiselius. She found that not only T-1824 but also T-1836, D-1824 and D-1836 combine entirely with the albumin fraction when these dyes are mixed with plasma or serum. She also found that the dye-albumin complexes differed strikingly in their tendency to dissociate when exposed to a cellophane surface, as shown by the staining of the cellophane. In the case of T-1824 in serum, none of the dye was transferred to the cellophane after 24 hours. D-1824 stained the strip slightly, whereas T-1836 and especially D-1836 left the strip deeply stained. Comparison of these observations with the time-concentration curves in figure 1 suggests that the differences in the rates at which the dyes leave the circulation are determined by the differences in the strengths of the bonds which the dyes form with the albumin. Furthermore it would appear that in the case of the dye T-1824 the binding is essentially equivalent to a tagging of the albumin.

According to Smith (1925) who carried out extensive investigations with brilliant vital red, a dye which in its behavior closely resembles T-1824, the process of elimination involves 1, diffusion from the plasma; 2, temporary storage in phagocytes and reticulo-endothelial system, and finally 3, excretion of the dye by the liver. The final process, excretion by the liver, must of course sever the dye-protein bond. Does this occur also during phagocytosis? Information concerning the mechanism by which the dye-albumin bond is severed in the body would be extremely desirable. The fact that Smith found a striking similarity in the dye and protein content of lymph from various regions may be regarded as evidence that the dye-protein bond is still intact in the tissue fluids and in the lymph.

One problem which must be reconsidered in the light of the preceding evidence is that of choosing among the linear, semilog and square root plots for determining the initial concentration of T-1824 by extrapolation. The test by plotting is unfortunately not always critical because of experimental variations in the data. That these variations need not be great in order to leave the issue in doubt may be readily seen by comparing the results of plotting a theoretical time-concentration curve in the three ways as shown in figure 4.

A straight line was arbitrarily drawn on the semilog plot in figure 4B to represent a loss of 15 per cent of the dye in 60 minutes. Values taken from this line are shown as hollow circles on the linear and square root plots in figures 4A and 4C. It will be noted that the linear plot of these data is so nearly a straight line that extrapolation on that assumption gives an initial concentration which is practically the same as that shown in the original semilog plot. On the square root plot, however, the values lie on a distinct curve. But one thing

must be noted, that if one takes into account only the points from 10 minutes to 90 minutes the deviation from a straight line is not great. Hence if one were dealing with actual observations in which the 10-minute value was slightly high because of incomplete mixing the plotted data might possibly be interpreted as a straight line on a square root plot. However the adoption of this interpretation as suggested by King, Cole and Oppenheimer (1943) implies that dye is lost rapidly during the mixing period, the rate approaching infinity at the time of

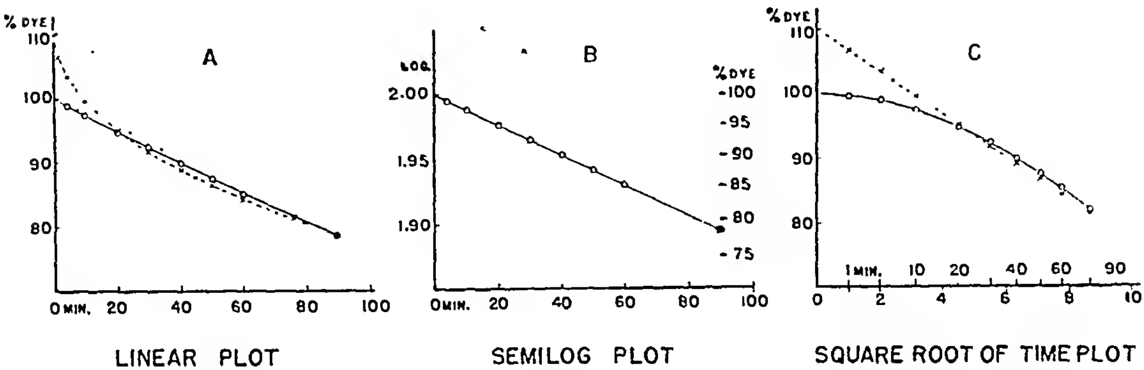

Fig. 4. Showing the curves obtained by transposing values from a straight line on a semilog plot (B) to a square root plot (C) and a linear plot (A). It will be noted that the *initial concentration* obtained by extrapolating the curve in (A) on the assumption that it is a straight line would differ only slightly from 100 per cent, whereas "straight line extrapolation" of (C) gives a value of 110 per cent.

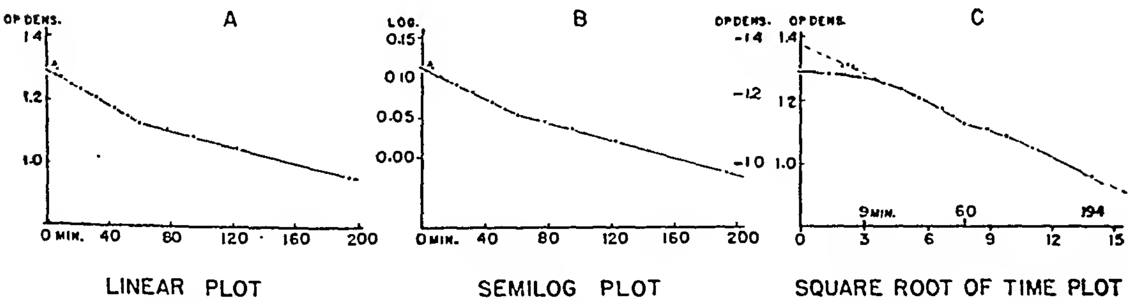

Fig. 5. Linear (A), semilog (B) and square root of time (C) plots of a typical time-concentration curve of T-1824 in a normal dog. "Straight line extrapolation" in (A) gives an initial concentration of 1.29; in (B) 1.30; in (C), 1.38.

injection (see broken line in fig. 4A). There is no valid evidence of such a rapid initial loss of T-1824.

From a critical examination of various data on semilog and square root plots we have concluded that during the first hour after injection the time-concentration curve is more precisely described by a logarithmic function than by a square root function. An illustration is given in figure 5. Both the linear and semilog plots of these data apparently give a straight line from 6 to 60 minutes after the injection, whereas the square root of time plot during the same interval is slightly curved. It should be pointed out also that in the square root plot of

the average curve for T-1824 in figure 3 there is a suggestion of a similar curvature which does not appear in the semilog plot of the same data in figure 2.

The logarithmic character of the disappearance curve of T-1824 is also in accord with the fact that this dye is firmly bound to the albumin. During the time when the disappearance rate is being determined, both the plasma volume and the plasma protein concentration presumably remain unchanged. Under these conditions the albumin must escape from and be returned to the circulating plasma at a constant rate. If the dye leaves the circulation only in combination with the albumin, then it follows that the amount of dye which escapes in unit time must be a constant fraction of the total amount of dye remaining in the blood. This is the basic condition for an exponential disappearance function and therefore the time-concentration curve should give a straight line on a semilog plot. That it does so seems to be shown by figures 2 and 5. However, when the dye begins to re-enter the circulation by way of the lymph, the same logarithmic function could not be expected to fit the data. Time-concentration curves of T-1824 on normal dogs regularly show a break. In the curve shown in figure 5 the break occurred at one hour, but as a rule it appears about two hours after the injection of the dye. Similar conclusions regarding the time-concentration curve have been reached by Hemingway, Scott and Wright (1935) in a study with the dye, water blue, and by Price and Longmire (1942) with T-1824.

The question may be raised as to why the time-concentration curves of trypan blue and niagara sky blue do not also follow a semilog function (see fig. 2). This clearly means that the mechanism by which these dyes escape from the plasma is not the same as for T-1824, and indeed certain facts already mentioned bear this out. The cellophane staining test (Rawson, 1943) shows that trypan blue and niagara sky blue are not as firmly bound to the plasma albumin as T-1824, and one would therefore expect that vital staining might be a large factor in their removal from the plasma. Initial loss of dye by staining probably accounts for the fact that these dyes give higher values for plasma volume (table 2). Furthermore, they escape into the urine, a path of excretion not available to T-1824.

It may be noted finally that one implication of the preceding discussion is that the rate of disappearance of T-1824 during the first hour after the injection is a measure of the rate of exchange of albumin. If this be true, the dye is obviously a convenient tool for studying changes in the local or overall "permeability" of the vascular bed to albumin.

CONCLUSIONS

From a correlation of the disappearance rates of T-1824, T-1836 (trypan blue), D-1824 (niagara sky blue 6B), and D-1836 (niagara sky blue) with Rawson's (1942) studies of dye-protein binding, it is concluded that the difference in disappearance of these dyes from the bloodstream is determined by the strength of the bond which is formed between the dye and plasma albumin. Although the dyes are closely related structurally they exhibit remarkable differences in behav-

ior (see fig. 1 and table 2) which are clearly related to their differences in structure (see Discussion).

The various lines of evidence which are cited suggest that T-1824 is so firmly bound to the albumin that the disappearance rate of this dye during the first hour after injection is a measure of the rate of escape of the circulating albumin. The disappearance curve of the dye should then follow a semilog function. This prediction is in our opinion confirmed by a critical examination of time-concentration curves obtained on normal dogs (figs. 2 and 5).

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THE BINDING OF T-1824 AND STRUCTURALLY RELATED DIAZO DYES BY THE PLASMA PROTEINS

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The purpose of the present study was to find out if the differences in disappearance rates of T-1824, trypan blue, niagara sky blue and niagara sky blue 6B depend upon the binding of these dyes by the plasma proteins (Gregersen and Rawson, 1943). The affinity of the various dyes for the plasma proteins was investigated: 1, with the electrophoresis method of Tiselius (1937); 2, by the ultracentrifuge; 3, by the effects of the plasma proteins upon the spectral absorption of the dyes, and 4, by a cellophane-staining test.

ELECTROPHORESIS EXPERIMENTS. The protein solutions were prepared for electrophoresis as follows: 4 cc. of serum or plasma were diluted with 12 cc. of a 0.2 M phosphate buffer, varying in pH from 7.40 to 7.60, and containing 0.15 M sodium chloride. The mixture was dialyzed at 5°C. in a cellophane bag for two or more days against two liters of the buffer. The buffer solution was changed at least once during the dialysis period. The dye was added from stock solutions before dialysis.[2]

Figure 1a shows the electrophoretic pattern of normal citrated human plasma. The pattern in figure 1b is produced by the same plasma containing T-1824 at a concentration of 0.004 per cent. The light absorption of the dye causes a well-defined shaded area. The fact that the shading begins with the ascending albumin boundary and ends with the descending albumin boundary demonstrates that the dye migrates entirely with the albumin. The same results were obtained with trypan blue, niagara sky blue, niagara sky blue 6B and also with brilliant vital red. In dog serum, as in human plasma, all of the dyes also migrated entirely with the albumin fraction. At the close of each dye-plasma experiment the protein boundaries were pushed by clockwork and plunger in order to separate the alpha, beta and gamma globulins on the descending side from the rest of the solution. Chemical and spectrophotometric examination of the globulin solution showed the presence of protein and the absence of dye.

The electrophoretic pattern of human plasma containing 0.098 per cent T-1824 (after dialysis) showed the dye boundary to begin with the ascending albumin boundary and to end with the descending beta globulin boundary. Hence if sufficient dye is present it will be bound by the alpha and beta globulins as well as by the serum albumin. The descending gamma globulin was free of dye. At the end of four hours of electrophoresis, the fractions listed in table 1 were

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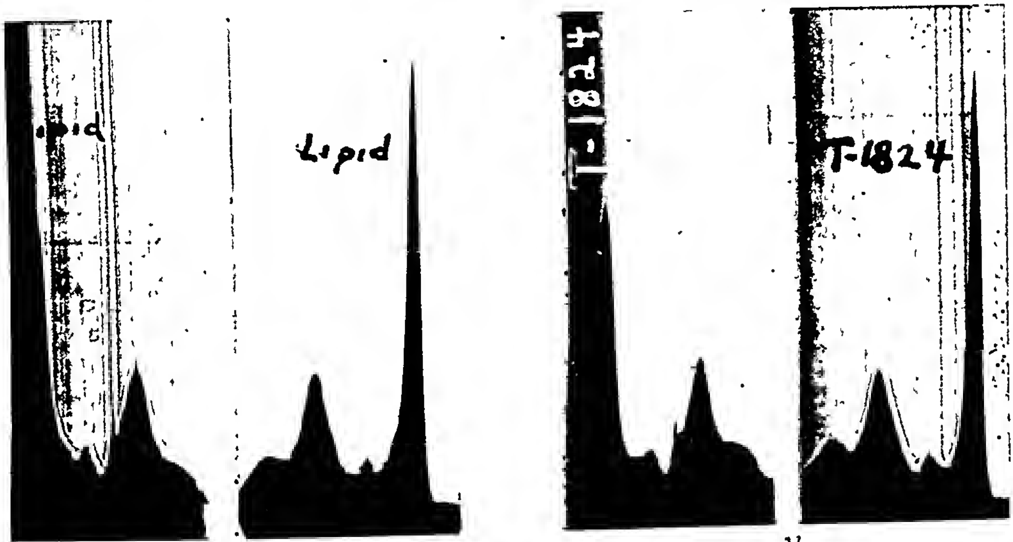
[2] Since trypan blue and niagara sky blue stained the cellophane bags during dialysis it was necessary to add these dyes to the plasma samples after dialysis.

separated. The concentrations of dye and protein in the different fractions were obtained by the König-Martens spectrophotometer and micro-Kjeldahl determinations. It should be noted that the ratio of T-1824 to albumin in the albumin fraction was approximately eight moles of dye per mole of albumin.

Experiments with T-1824 and electrophoretically separated human serum albumin. T-1824 was added to a sample of electrophoretically separated human serum albumin in high concentration. The final concentration of dye (after



1*a* 1*b*

Fig. 1. Electrophoretic pattern of normal human plasma. *a*, without, and *b* with T-1824 added.[3]

TABLE 1

The distribution of T-1824 (0.098 per cent) in human plasma after electrophoresis

ELECTROPHORETIC SAMPLE	PROTEINS PRESENT	PER CENT DYE*	MGM. N PER CC	MOLES OF DYE† / MOLES OF PROTEIN
1: Before electrophoresis	Albumin and globulins	0.098	1.74	
2	Albumin	0.063	0.76	8.3
3	Globulins	0.006	0.41	5.0
4	Gamma globulins	0.00	0.086	

* See page 710 for method of calculating dye concentration.
† Molecular weights used in calculating ratio: Albumin 70,000, Globulin 150,000, Dye 960.

dialysis) was 0.019 per cent in 0.098 per cent albumin, the ratio of dye to albumin being fourteen moles of dye per mole of albumin. After four hours the T-1824 migrated ahead of the albumin on the ascending side. Table 2 gives the mobility of the albumin before and after the adding of T-1824. The mobility of albumin is not affected by T-1824 at a concentration of 0.004 per cent (ratio of

[3] According to Blix, Tiselius, and Svensson (1941) the shading beginning and ending with the beta globulin boundaries is caused by the presence in the plasma of the lipids which migrate with the beta globulin.

dye to albumin, 1 to 28) but if the dye concentration is increased to 0.019 per cent (ratio of dye to albumin, 14 to 1) the mobility is increased from 5.0×10^{-5} to 7.0×10^{-5}. The significance of this change in mobility will be discussed later.

Electrophoresis of T-1824 in globulin solution. A 2.3 per cent globulin solution prepared from normal human plasma by the method of Howe (1921) showed the presence of two protein fractions corresponding in mobilities to alpha and gamma globulin. When T-1824 was added to this globulin solution to a concentration of 0.002 per cent, the dye migrated wholly with the alpha globulin.

ULTRACENTRIFUGE AND DIFFUSION EXPERIMENTS. The observation that the dyes migrate preferentially with the albumin fraction has been confirmed by the sedimentation of T-1824 with serum albumin in the ultracentrifuge. The molecular weights of the albumin-dye complexes in solutions of various dye-albumin ratios were calculated from the sedimentation rates and diffusion constants according to the formula of Svedberg (Svedberg and Pedersen, 1940).

TABLE 2

The effect of a high concentration of T-1824 on the mobility of human serum albumin

	CONCENTRATION OF		MOLES OF DYE / MOLES OF ALBUMIN	pH	MOBILITY OF ALBUMIN ($\mu \times 10^5$)	
	Albumin mgm/cc. ($7.0 \times N$)	Dye per cent			A*	D*
Serum albumin	0.98	no dye		7.45	5.00	5.50
Serum albumin plus high concentration of T-1824	0.98	0.019	14	7.48	7.03	7.65

* A is the mobility of the ascending boundary, D the mobility of the descending boundary.

$$M = \frac{RTS}{D(1 - Vp)}$$

where $R = 8.212 \times 10^{-7}$

$T = 293°$ absolute
$D =$ Diffusion constant at 20°C.
$S =$ Sedimentation rate at 20°C.

The sedimentation rates were determined in an air driven ultracentrifuge at 800 revolutions per second (158,000 × gravity) and at 24° to 31°C. The diffusion constants were determined at 2° or 8°C. in an electrophoresis cell and diffusion curves were obtained at intervals up to 75 hours on photographic plates using the Longsworth schlieren scanning method (1939). The sedimentation rate and diffusion constant of electrophoretically separated human serum albumin in phosphate buffer (pH 7.49) were determined under the following conditions: 1, without dye; 2, containing 0.00049 per cent T-1824, and 3, containing 0.038 per cent T-1824 (see table 3).

In each of the three sedimentation experiments only one boundary was present. When dye was present it sedimented with the albumin leaving the supernatant buffer colorless. Table 3 lists the data obtained. It should be noted that in a preliminary experiment in which T-1824 in 0.004 per cent concentration in phosphate buffer was centrifuged at 900 revolutions per second, no sedimentation was observed after $1\frac{1}{2}$ hours.

Discussion of electrophoresis and ultracentrifuge data. The binding of the dyes by the plasma proteins is clearly demonstrated in the electrophoresis experiments (fig. 1). This is further borne out in the case of T-1824 by the ultracentrifuge studies. The dyes, T-1824, niagara sky blue 6B, trypan blue, niagara sky blue and brilliant vital red in 0.004 per cent concentration in plasma are wholly and preferentially bound by the albumin fraction.

The experiments show that if the dye concentration is increased sufficiently, the acid dyes may also be bound by the globulin fraction. Furthermore when

TABLE 3

*The molecular weight of human serum albumin before and after adding T-1824 as determined in the ultracentrifuge**

	CONCENTRATION OF		MOLES OF DYE / MOLES OF ALBUMIN	DIFFUSION CONSTANT AT 20°C. × 10[7]	SEDIMENTATION RATE AT 20°C. × 10[13]	MOLECULAR WEIGHT
	Dye per cent	Albumin mgm/cc. (N × 7.0)				
Serum albumin................	0	5.60	0.0	6.3	4.56	70,300
Serum albumin plus dilute T-1824......................	0.00049	5.60	0.067	5.9	4.32	71,000
Serum albumin plus concentrated T-1824..............	0.038	4.97	4.8	6.0	5.13	83,000

* The serum albumin used in these experiments was electrophoretically separated from human plasma.

T-1824 is added to a solution of alpha and gamma globulins it migrates preferentially with the alpha globulin.

The data make it possible to estimate the approximate number of molecules of T-1824 which can be bound by a molecule of albumin at physiological pH and salt concentration. This dye in plasma migrates only with the albumin fraction unless the ratio of dye to albumin is increased beyond eight moles of dye per mole of albumin. When T-1824 is added to an albumin solution so that the ratio of dye to albumin is 14, some of the dye slowly leaves the albumin during electrophoresis. This would indicate that albumin can bind somewhat less than 14 molecules of dye per molecule of albumin. The ultracentrifuge data show that T-1824 is bound by the albumin in increasing proportions as the concentration of dye is increased, and this is also indicated in the electrophoresis experiments by the effect of T-1824 in low and high concentrations on the mobility of human albumin (table 2). The increase in mobility of human albumin containing relatively high concentrations of T-1824 at pH 7.4 may be explained by the assumption that as T-1824 is attached to the albumin it prevents the dissociation

of some of the basic groups. This shows that when a sufficient amount of dye is bound to albumin the isoelectric point of the albumin-dye complex is significantly lower than that of albumin.

ABSORPTION SPECTRA OF THE DYES IN PROTEIN SOLUTIONS. The absorption spectra for the dyes (T-1824, niagara sky blue 6B, trypan blue and niagara sky blue) were determined by Gregersen and Gibson (1937) in water, 0.9 per cent sodium chloride and plasma. The curves of T-1824 and niagara sky blue 6B in plasma show an asymmetry about the point of maximum absorption which is not present in the water solutions. This suggests that the effect of albumin and globulin on the absorption spectra of these dyes might be related to the binding of these dyes by the proteins.

The observations were made with a König-Martens visual spectrophotometer. The albumin solutions prepared by ammonium sulfate precipitations were found by electrophoretic analysis to be free of globulin. Howe's method (1921) for preparing the globulin solution was used, and according to electrophoretic analysis, this solution contained only alpha and gamma globulins. The protein solutions were dialyzed (5°C.) for three days against running water, and for two days with several changes of distilled water or phosphate buffer (pH 7.40).

Figure 2 shows the absorption curves of the four blue dyes in human plasma, human albumin and human globulin solutions. In each instance the shape of the curve obtained in albumin solutions (1.0 per cent) was the same as that in plasma. Globulin solutions in concentrations as great as those occurring in plasma depressed the optical density and shifted the maxima of the dyes from that observed with buffer (Gregersen and Gibson, 1937), but did not produce the asymmetry as seen in the plasma curves of T-1824 and niagara sky blue 6B.

That the changes in the absorption spectra of the dyes produced by plasma are caused mainly by the albumin fraction is demonstrated by the results presented in figure 2. The effect of various concentrations of electrophoretically pure albumin were therefore studied in greater detail and the results are given in figure 3. Figure 4 shows the change of optical density at the maximum for the four dyes as plotted against the ratio, moles of albumin per mole of dye.

Comparison of the optical density at the point of maximum absorption of solutions of 0.001 per cent and 0.002 per cent T-1824 in various concentrations of albumin showed that the Lambert-Beer Law holds in this range of concentration when protein-dye solutions of the same ratio of protein to dye are compared. This was the range of concentration in which the optical density measurements were made.

From the above and figure 3 it is evident that within certain ranges of dye and albumin concentration the concentration of dye cannot be determined unless the ratio of dye to albumin is known. However, by plotting a full curve for the unknown solution (520 mμ to 640 mμ) and by matching this curve in respect to *both shape and point of maximum absorption* with those obtained on solutions of known dye-albumin concentration, and relating the optical density at the point of maximum absorption as in the equation below, the true concentration of dye in the unknown can be determined.

$$\text{Per cent concentration of dye in unknown} = \frac{\text{O.D. of unknown}}{\text{O.D. of the 0.002 per cent curve}} \times 0.002 \text{ per cent}$$

A check on the ratio of dye to protein obtained by this method was made by a micro-Kjeldahl determination of the protein concentration and in all experiments the two values checked quantitatively.

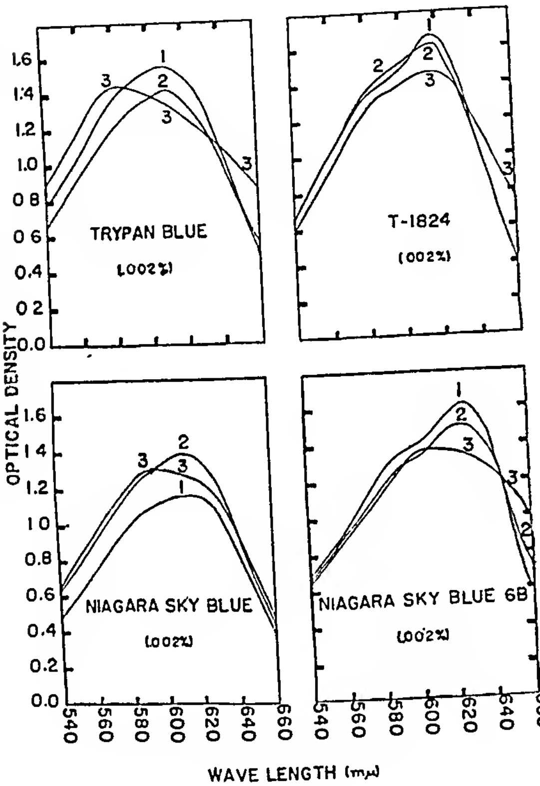


Fig. 2

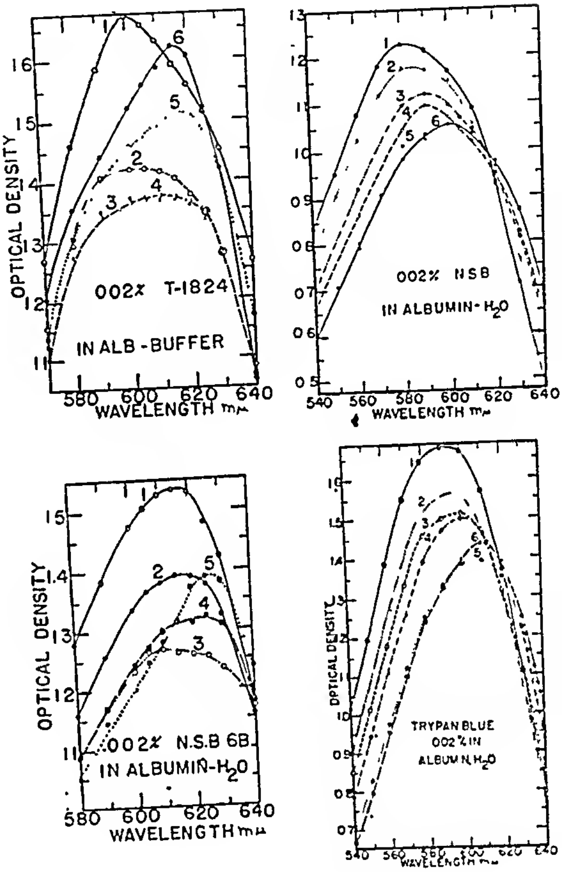


Fig. 3

Fig. 2. Absorption spectra of the dyes in 1, plasma; 2, plasma albumin, and 3, plasma globulin.

Fig. 3. The effect of varying the albumin concentration on the absorption spectra of the four dyes (0.002 per cent). The curves for T-1824 were obtained in phosphate buffer at pH 7.3; the curves for the other three dyes were obtained in water. The moles of albumin per mole of dye were as follows: T-1824 (1) 0.000 (2) 0.057 (3) 0.096 (4) 0.11 (5) 0.48 (6) 4.8; niagara sky blue 6B (1) 0.00 (2) 0.099 (3) 0.14 (4) 0.20 (5) 0.99; trypan blue (1) 0.00 (2) 0.053 (3) 0.096 (4) 0.24 (5) 0.48 (6) 7.7; niagara sky blue (1) 0.00 (2) 0.10 (3) 0.14 (4) 0.20 (5) 0.50 (6) 7.9.

Discussion of spectral absorption experiments. The observations that the changes in the absorption curves of the four blue dyes produced by plasma are caused mainly by the albumin fraction (fig. 2) is in agreement with the preferential binding of these dyes by the albumin as observed in the electrophoresis studies. It should be noted that Robinson and Hogden (1941) have recently found that the albumin fraction of serum is also mainly responsible for the

changes produced by a serum-buffer system on the absorption spectrum of phenol red.

Definite differences were observed among the four dyes when the effect of various albumin concentrations on the absorption spectra were compared (figs. 3 and 4). A shift of about 20 mμ to the red end of the spectrum was observed with all the dyes in albumin solutions. This would indicate a dampening of the bond energies of the dye as binding with protein takes place. As the albumin concentration was increased in the presence of a constant amount of dye (0.002 per cent) the optical density at the point of maximum absorption of all four dyes fell (fig. 4). The optical density of T-1824 fell to a minimum value when the ratio moles of dye per moles of albumin was 11. The optical density rose when the concentration of albumin was increased and at high albumin concentrations

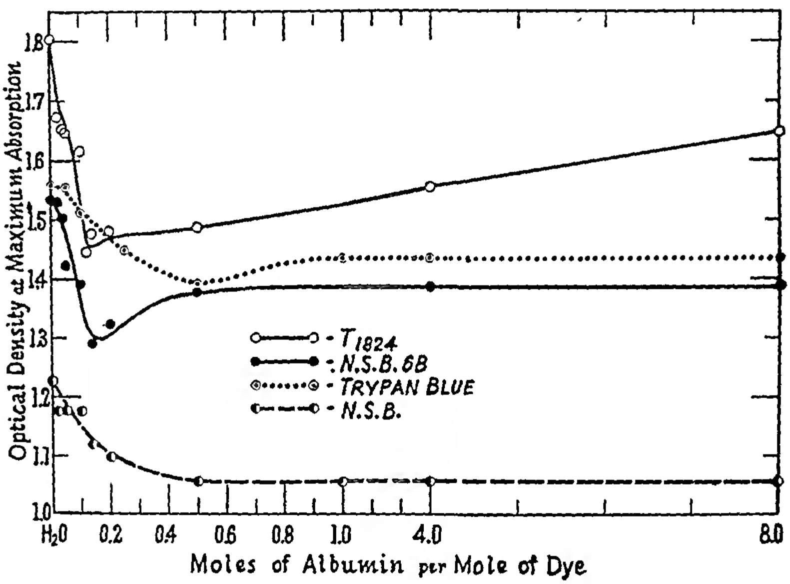


Fig. 4. Variation in optical density at maximum absorption with increases in albumin—dye ratio.

reached that observed in water. A similar variation in optical density was obtained with niagara sky blue 6B. The optical density fell until the ratio, moles of dye per moles of albumin was 8.3 and then increased in value. The rise in optical density from the point of maximum depression was small in trypan blue (fig. 4). Efskind (1940) has observed a similar result for trypan blue using dog plasma. No rise was observed with niagara sky blue.

The experiments suggest that the depression of the optical density by albumin represents binding of the dye, and the continuous fall in optical density with increasing protein concentration represents the conversion of free dye to dye-protein.[4] According to this concept the minimum value of the optical density

[4] The depression in optical density produced by albumin cannot be explained as a salt effect since Gregersen and Gibson (1937) found that sodium chloride produced no depression of the optical density in plasma solutions.

should represent the point at which the maximum number of dyes are bound per molecule of albumin. This interpretation is supported by the electrophoretic studies with T-1824, in which it was observed that the maximum number of moles of dye that could be bound by a mole of albumin lay between 8 and 14. When the concentration of albumin is further increased, the optical density rises as the composition of the dye-protein complex changes from a high ratio of dye to protein to lower ratios. It should be noted that in plasma volume determinations the effect of changes in albumin concentration on the optical density of T-1824 does not have to be considered since the ratio of moles of albumin per moles of dye is greater than 10. This fact is also evident from figure 10 of Gregersen and Gibson (1937).

Cellophane staining test. Water solutions of the dyes do not stain cellophane, but if a trace of salt is added to the system, the dyes are immediately and completely deposited on the cellophane. The dye so deposited cannot be removed by washing with water. When 0.002 per cent solutions of these dyes in plasma were placed in cellophane bags and left in the icebox dialyzing against buffer, it was found at the end of a month that the bags containing niagara sky blue and trypan blue were heavily stained and the respective plasma solutions colorless, while the T-1824 and niagara sky blue 6B bags remained unstained and the dyes were still in solution. These observations are striking in view of the fact that niagara sky blue and trypan blue escape from the circulation much more rapidly than T-1824 or niagara sky blue 6B (Gregersen and Rawson, 1943). Since the relative concentration of dye to protein was the same in all bags, and since the electrophoresis experiments have shown that the dyes in this concentration are wholly bound by the albumin, the above observation indicates a difference in the degree of dissociation of the various dye-protein complexes in the presence of a cellophane surface.

The staining of cellophane by the four blue dyes in various concentrations of human albumin was therefore studied more carefully. For each dye a series of dye-albumin solutions were prepared by adding 0.2 cc. of 0.04 per cent dye to 3.8 cc. of albumin giving final concentrations of albumin ranging from 0.17 to 4.20 grams per cent. A strip of cellophane (approximately 6 x 12 mm.) was immersed in each tube and observations were made at intervals up to twenty-four hours.

The albumin solutions used in these experiments had been dialyzed against 0.02 M phosphate buffer, pH 7.4, and contained 0.15 M sodium chloride. The pH of the dye-protein solutions lay between 7.3 and 7.4 as determined by the glass electrode. An abbreviated protocol of the experiments with the four blue dyes in albumin solutions is given in table 4.

From the protocol, table 4, it is apparent that there is a difference in the affinities of the dyes for albumin in the presence of cellophane. Nia[illegible] sky blue-albumin solutions stained the cellophane strips within 1 to 2 hou[illegible] tubes where the ratio of albumin to dye was 3 to 1. T-1824, in [illegible] albumin having a ratio of one or more molecules of alb[illegible] not stain cellophane strips in a period of twenty-four [illegible]

blue 6B albumin solutions stained the cellophane to only a slightly greater extent than the T-1824 albumin solutions. Deeper staining was observed with trypan blue-albumin solutions and the deepest staining occurred with niagara sky blue.

DISCUSSION. The progressive difference in the affinities of the four blue dyes for albumin in the presence of cellophane is apparent from these tests. Since the electrophoresis experiments have shown that these dyes, in the concentrations employed in the disappearance studies, are wholly bound by the albumin fraction of plasma these differences in their affinities for albumin are significant. It will be noted that the relative affinities of the dyes for albumin bear an inverse relation to their disappearance rates from the circulation. T-1824 has the highest affinity and the lowest disappearance rate (8 to 10 per cent per hour), while niagara sky blue has the lowest affinity and the highest disappearance rate (54 per cent per hour) as shown by Gregersen and Rawson (1943).

TABLE 4

Protocol of cellophane staining test

ALBUMIN CONCENTRATION (N × 7.0)	MOLES OF ALBUMIN PER MOLE OF DYE	T-1824 (0.002 per cent)		N.S.B.6B (0.002 per cent)		Trypan blue (0.002 per cent)		N.S.B. (0.002 per cent)	
		Time in hours							
		4	24	4	24	4	24	4	24
mgm./cc.									
4.20	2.9	−	−	−	−	−	+++	+++	++++
1.40	0.96	−	−	−	+	+	++++	+++	++++
0.70	0.48	−	±	−	++	+++	++++	+++	++++
0.28	0.19	−	++	−	++	++++	++++	++++	++++
0.20	0.13	−	++	±	+++	++++	++++	++++	++++
0.17	0.12	−	+++	±	+++	++++	++++	++++	++++

−, No staining; ±, cellophane same shade as solution; +, cellophane darker than solution.

NSB6B = niagara sky blue 6B; NSB = niagara sky blue.

SUMMARY AND CONCLUSIONS

The electrophoresis experiments show that T-1824, niagara sky blue 6B, trypan blue, and niagara sky blue in serum or plasma are wholly bound by the albumin fraction when they are present in low concentrations, i.e., about 0.004 per cent or less. This no longer holds, however, when the dye concentration exceeds certain limits representing the binding capacity of the albumin. The evidence indicates that each mole of albumin can bind a maximum of 8 to 14 moles of T-1824.

Ultracentrifugation of serum albumin solutions containing 5 moles of T-1824 to 1 mole of albumin also demonstrates that the dye is bound by the protein. The dye comes down with the albumin forming a single boundary leaving the supernatant buffer solution colorless.

Addition of plasma to aqueous solutions of the dyes shifts the point of maximum absorption toward the red end of the spectrum and changes the contour of the spectral absorption curve (Gregersen and Gibson, 1937). These effects are

caused mainly by the albumin fraction (see fig. 2). There is however some difference in the effect of albumin on the absorption of the four dyes. With the two dyes, niagara sky blue and trypan blue, the optical density falls as the albumin concentration is increased to the point where its molecular concentration is $\frac{1}{2}$ that of the dye. Further increase in the albumin concentration is without much effect. With T-1824 and niagara sky blue 6B the maximal effect of the addition of albumin is reached at much lower concentrations, approximately $\frac{1}{10}$ moles of albumin per mole of dye, and the optical density then increases as the albumin concentration is raised still further (figs. 3 and 4). This ratio for the maximum number of T-1824 molecules bound by a molecule of albumin is in agreement with the value obtained from the electrophoretic studies.

The cellophane staining tests reveal differences in the affinities of the four dyes for albumin. There is a direct relationship between the rates at which these four dyes leave the circulation (Gregersen and Rawson, 1943) and their tendency to stain cellophane (see table 4). Thus although all four dyes are preferentially bound in the plasma by albumin an explanation for their different disappearance rates is found in their different affinities for albumin.

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AN EXPERIMENTAL STUDY OF FLOW PATTERNS IN VARIOUS PERIPHERAL ARTERIES[1, 2]

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During experimental studies concerning the accuracy of the thermostromuhr method for measuring blood flow (1, 2), the existence of back flow in a flow pattern was found to constitute a source of very large error in the determination of directional changes in, or quantitation of, the mean rate of flow through a vessel. It therefore became a matter of considerable interest to know in which vessels, of experimental animals, backflow normally exists and under what conditions it may appear.

Utilizing different methods, the studies of other investigators indicate, for the anesthetized dog, the normal presence (3) or absence (4, 5) of backflow in the carotid artery, its absence in the femoral (5, 6) and left coronary arteries (5), but its frequent occurrence in brachial and femoral arteries of humans (7, 8). Since experimental studies of flow patterns are not only limited but are contradictory in certain of the findings and since our own work has shown that coronary flow patterns may exhibit various degrees of backflow, an extension of the study was made to include the velocity curves of a number of peripheral arteries. These were recorded by an orifice type flow-meter (9, 10) which is adaptable to use in various body regions. Tests have demonstrated its ability to respond to frequencies of 90 to 120 D.V. per second in the blood stream, which characteristic permits adequate recording of all but the extremely rapid phasic changes in the rate of flow through the arterial vessel.

Possession of such flow patterns not only permitted selection of those arteries in which thermostromuhr flow values would be vitiated by the presence of backflow, but, of more importance, the flow curves taken together with their corresponding simultaneously recorded intravascular pressure curves afforded an opportunity for a comparative study of the flow pulses in heteronymous arteries. As the work progressed, a general system of analysis and interpretation of flow pulse patterns was attempted. Such patterns and their analysis, in so far as experimental data permit, are presented here.

Procedure and methods. Dogs weighing from 10 to 22 kgm. were used. The artery under study was exposed under local (procaine 2 per cent, or morphine and procaine) or general (sodium pentobarbital, sodium amytal, or ether) anesthesia; heparin, 100 units per kgm. and pontamine fast pink, 150 mgm. per kgm. were administered intravenously. An orifice-type flow meter, previously de-

[1] The expenses of this investigation were defrayed to a large extent by a grant from the Commonwealth Fund.

[2] A preliminary report of a part of this work was made before the American Physiological Society at the Chicago meeting, April 1941, and at the Boston meeting, April 1942.

signed by Gregg and Green but more recently modified by one of us (R. E. S.), was inserted between, and tied to, the cut ends of the vessel and then buried in the surrounding tissues. The existing "normal"[3] flow pattern together with the lateral blood pressure (11, 12) were recorded optically. The modifications in the flow meter were: 1. All tubular connections were made as large in diameter and as short as feasible. 2. All stop cocks were removed and the length of the flow tube was shortened to about 1.8 cm. 3. In place of the removable disc with a fixed orifice, the constriction necessary for the desired sensitivity was obtained by appropriate manipulation of an adjustable rounded stud screw, the central end of which protruded into the flow tube lumen between the lateral pressure apertures. These modifications insured not only a minimum of instrumental

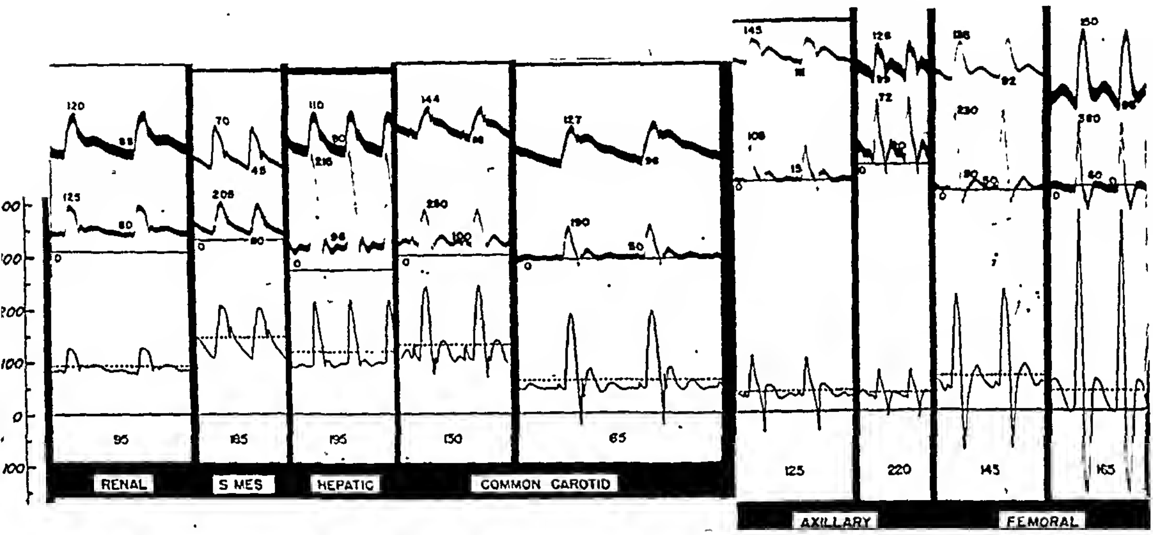

Fig. 1. Reproductions of original pressure (upper) curves and flow (middle) curves as recorded in the various arteries indicated. Lower curves are rectified reconstructions of original velocity curves. Pressure values (mm. Hg) and flow rates (cc. per min.) indicated by numbers on respective curves. Heart rate indicated by numbers at bottom of each segment. O, zero flow. Ordinate scale, flow in cubic centimeters per minute.

damping, but also facilitated the adjustment of the apparatus to any desired sensitivity during the experiment (cf. fig. 5 for details).

In 52 dogs flow patterns were obtained, usually in one and occasionally in two of the following arteries: renal, hepatic, superior mesenteric, common carotid, axillary, and femoral.

Normal patterns. Flow curves, characteristic for their respective arteries, are presented in figure 1. For clearer visualization of the flow rate/time relationship in a given curve and to permit comparisons among difference curves,

[3] In this instance, "normal" refers to those patterns recorded in dogs which have undergone only those procedures necessary for the recording of flow patterns, namely, administration of the above anesthetics and anticoagulants, and isolation of the artery and insertion of the orifice meter.

the recorded patterns have been rectified[4] and redrawn to a linear ordinate scale. These will be considered in relation to each other and to their respective pressure pulses.

Although flow curves have been obtained from a number of different dogs and recorded with different heart rates and blood pressures, flow patterns obtained in arteries supplying different body regions are subject to comparison on a common etiological basis. Examination of the large number of flow curves which have been recorded in different arteries has made possible the identification of certain distinguishing characteristics which, although easier to recognize than to describe, will be collectively examined in the following comparative and descriptive analysis.

On visual inspection, the flow patterns as recorded[5] in peripheral arteries are composed of a series of waves whose directional changes have a qualitative correspondence with gradient changes in the simultaneously recorded intravascular pressure pulses. However, the quantitative relationship of phasic rate of flow to pressure varies widely within a single cycle.

Since flow velocity varies with the differential pressure existing at the site of the flow meter, the similarity in contour of the flow and applied pressure pulses constitutes one criterion for the comparison of flow curves from different arteries and is also the basis for evaluating the influences of inherent anatomical differences among the vascular beds, the effects of which will be considered later. Certain patterns, such as the superior mesenteric and renal, retain a rather well rounded and sustained systolic portion in relative conformity to that of the pressure pulse; that of the hepatic and common carotid is less rounded while the axillary and femoral have a sharp systolic spike. The maximal pulse amplitude is seen to be small in the case of the renal and axillary patterns, somewhat larger in the superior mesenteric and hepatic, while that of the common carotid and particularly femoral patterns is quite large. Back flow is frequently present in the common carotid and consistently found in axillary and femoral patterns but has not been observed in the "normal" renal, superior mesenteric, or hepatic patterns. However, the striking feature which allows a differentiation of all the curves is the variability of the early diastolic rate of flow with respect to the presystolic rate. A comparison of this relationship with that of the corresponding early diastolic and presystolic points on the pressure curve reveals the superior mesenteric, renal, hepatic, common carotid, axillary and femoral patterns to have, in this respect, a progressively graded dissimilarity to their respective pressure curves.

[4] The original records exhibit a non-linear relationship of deflection to rate of flow in which the deflection varies exponentially with the rate of flow.

[5] The recorded patterns and rates of flow are regarded as only approximate and not exact representations of those flow changes which would have occurred had the flow meter not been inserted in the artery under study. In common with many flow meters the orifice-type meter limits flow through itself because of fluid friction within the meter unit. Hence, the amplitude of flow wave components and magnitude of mean flow changes presumably would have been slightly greater in the intact vessel than those which have been recorded and are presented here.

Flow patterns in some of the above mentioned arteries have been obtained with other methods and reported by previous investigators. Although agreeing in some respects, the patterns differ considerably from those presented here. Hewlett and Van Zwaluwenburg (7) made plethysmographic volume pulse recordings of the human forearm and arrived at flow velocity curves by differentiation of the volume curve. The presence of back flow was occasionally found normally, and invariably after nitroglycerine administration. The plethysmographic volume curves of Wright and Phelps (8) demonstrated the existence of back flow in the arterial supply of the human leg but no velocity curves were derived. Because of the difference in subjects used a common basis is not afforded for a comparison of their flow data with that presented here. Regardless of the preparation, however, it should be stressed that mean and phasic inflow values, as obtained with the plethysmograph become progressively diminished, beginning at that time when venous outflow is stopped (cuff is applied). Using the orifice meter, venous occlusion has been shown to diminish promptly the arterial inflow to the dog's leg, while at the same time augmenting markedly the normally existing back flow (13). Phasic flow records, obtained with plethysmographic method, should therefore be evaluated cautiously.

Machella (5), using a hot wire method, has presented velocity pulse curves from the femoral, carotid, and left coronary arteries of anesthetized dogs. These curves are grossly dissimilar to those shown above (and elsewhere (10, 19)) and none reveals the presence of a back flow component. Similarly, with the electromagnetic method, the carotid patterns obtained by Katz and Kolin (4) and the femoral patterns by Kolin (6) fail to exhibit back flow under normal conditions. However, Bergman (3), using the "stromborste," has published curves for the carotid of the rabbit which are quite similar to the carotid patterns (dog) presented here in figure 1.

The explanation for the pattern differences as obtained with the hot wire and electromagnetic recorders is not available. However, certain statements seem warranted. The frequency response of these instruments to changes in rate of blood flow has not been presented in the associated publications. The relatively smooth and rounded contours of the pulse waves obtained suggest that the recorded flow patterns[6] are appreciably damped representations of the actual intra-arterial velocity changes. This effect is presumably associated with the inherent characteristics of the instruments *as used*. The fact that "there is no lag in induction of an e.m.f." (Kolin, electromagnetic method (6)) does not justify the contention that "the blood velocity during each phase of the cardiac cycle is instantaneously indicated" on the graphic record. Thus, while it is granted that each of these methods may be capable of recording arterial flow

[6] It has been found that, with the orifice meter, very similar patterns can be produced rather easily by mechanical damping. If, for example, the tubes connecting the orifice tube to the optical recording unit are too long, or too small in diameter, a back flow component, known to exist in the flow pattern, may not be recorded at all or will be greatly reduced in magnitude. Similar error may follow a moderate constriction of an artery comparable to that which occurs with the application of a snugly fitting thermostromuhr unit, and which may occur when the electromagnetic unit is applied.

patterns, experimental tests of the response of the *entire* working instrument is known rapid fluctuations in flow velocity are needed before the validity of the recorded patterns can be established.

ANALYSIS AND INTERPRETATION. The foregoing peripheral patterns (fig. 1) reveal the existence of wide variations in volume, timing, direction and rate of flow. With the exception of the work of Hewlett and Van Zwaluwenburg, flow patterns have been considered primarily from a descriptive standpoint. While the recorded data for this and a companion paper (18) were being assembled and studied, a semi-quantitative method of analysis was evolved which was found to facilitate the recognition and identification of flow patterns of various peripheral arteries.

It became apparent early in the study that, for a given bed, the character of the venous outflow pattern was an important consideration in the analysis of the arterial inflow pattern. Venous flow patterns were recorded in the femoral, renal, and *high* in the external jugular veins under control conditions and following the injection of vasodilator drugs. The phasic variations[7] in the rate of venous flow were never found to be greater than 5 per cent of the mean flow value. Venous phasic flow studies found in the literature have been largely confined to the head circuit. Direct observations (14) have shown that flow pulsations in the capillaries and small veins of the rabbit's ear are almost nil.

These and our findings do not necessarily conflict with the observations of Burton-Opitz (15) and Holzlöhner (16) who found that the external jugular flow pattern is markedly pulsatile. Flow patterns very similar to those of Holzlöhner have been obtained with the orifice meter when the instrument was inserted into the lower portion of the vein (in the region of the supraclavicular fossa). Because of the proximity to the heart and the marked phasic changes in the recorded venous pressure, we concur in the belief (15, 16) that the pulsatile flow waves obtained in this portion of the vein are (central) cardiac in origin rather than (peripheral) waves transmitted through the capillaries. Our conception of the probable determinants of flow *pattern* and the method by which they were identified are illustrated in the following stepwise analysis of a sample flow pattern.

In figure 2 are shown reconstructions of a femoral arterial flow pattern and pressure curve, recorded as previously described for similar femoral patterns shown in figure 1. Under conditions of reasonable constancy the mean rate of arterial inflow during a given cycle is accepted as representing the existing mean rate of blood flow through the corresponding bed. This is indicated by the line *MM*. The areas representing volume flow, which lie above (*B*, *E*) and below (*A*, *C*, *D*, *F*) the mean flow line are equal by virtue of the mean position occupied by the mean flow line, *MM*. Since, aside from the comparatively

[7] Although these are conceivably remnants of the original flow pulse which has traversed the capillary bed, they are possibly artifacts resulting from generalized arterial impact phenomena, or more probably flow responses to cyclic fluctuations in the central venous head of pressure. In support of the last mechanism it was observed that simultaneously recorded venous pressure and mean flow waves underwent reciprocal directional changes.

slow respiratory fluctuations (17), the femoral venous flow pattern exhibits only very small cyclic variations and is essentially a smooth, "linear" flow curve, it corresponds very closely to the straight line *MM*, which is used as its graphic representation in this analysis. It therefore follows that from the point of recording arterial inflow to that of recording venous outflow (line *MM*), the flow pattern has been smoothed out, presumably by viscous resistance to flow and volume-elastic moderation. In the following portion of the analysis, the rela-

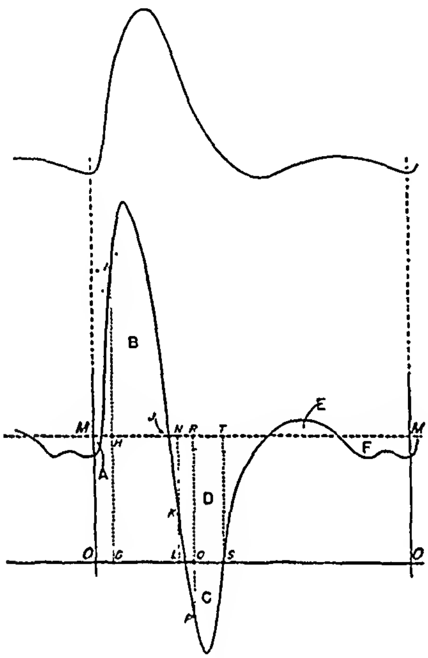

Fig. 2

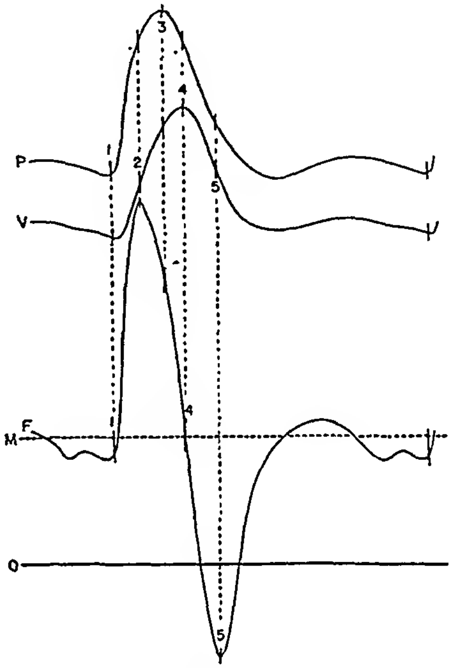

Fig. 3

Fig. 2. Upper curve—Reproduction of femoral arterial pressure pulse. Lower curve—Reconstructed flow pattern, rectified from original optical recording. Interrupted line *MM*, mean flow level. Line *OO*, zero flow level. Areas *B*, *E*, volume-elastic increments. Areas *A*, *C*, *D* and *F*, volume-elastic decrements. *C*, area of back flow. For other notations, see text.

Fig. 3. Same flow (*F*) and pressure (*P*) curves as shown in figure 2 with derived intravascular volume curve, *V*. *M* and *O*, same as in figure 2. Synchronous vertical intercepts on each curve, reading consecutively, are: 1, onset of flow and pressure rise; 2, maximal rates of forward flow and volume increase; 3, peak of applied pressure; 4, point of maximal intravascular volume; 5, maximal rates of back flow and volume decrease. Further explanation in text.

tionship of the pulsatile arterial inflow curve to its corresponding essentially "linear" venous outflow curve reveals the basic mechanism responsible for the recorded phasic variations in rate of inflow (flow pattern).

Since an arterial vascular tree is functionally an expansible chamber as well as a tubular conducting system, application of a pulsatile pressure head will give rise to a pulsatile change in the contained volume of that arterial tree. The significance of the volume changes may be illustrated in figure 2. The area

enclosed by the mean flow line (*MM*), zero flow line (*OO*), and the two intercepts marking the beginning and end of one cycle (*MMOO*) represents the volume of blood passing through the bed during a given cycle. Since the venous outflow pattern can be regarded as essentially a straight line (*MM*), the areas *B* and *E* above the mean flow line represent a certain volume of blood accommodated in the arterial tree *in excess* of that leaving the bed during the same time interval. Furthermore, the areas (*A*, *C*, *D*, *F*) below the mean flow line represent an *equal* volume of blood which the vascular tree does not accept during the same cycle as the necessary accompaniment to the accommodated excess. In fact, during the early systolic portion of the cycle the vascular tree has accommodated a volume greater than that which can run off peripherally during the late systolic and early diastolic portion, and a back flow of blood is recorded. In the flow curve illustrated, the amount of excess which cannot immediately participate in the peripheral run off is indicated as a definite back flow volume (cf. area *C*). However, as will be shown in a subsequent communication (18), as the mean flow through a bed becomes sufficiently large the systolic excess is compensated by a period of decreased forward flow rather than by a period of back flow. On the other hand, if the mean arterial inflow becomes smaller the volume of back flow per cycle is increased and the forward flow component is decreased. Under conditions in which the mean rate of flow is quite small the flow pattern may indicate the forward flow increment to be almost entirely canceled out by the back flow decrement. Thus, the pulsatile flow waves (fig. 2—*A*, *B*, *C*, *D*, *E*, *F*) which necessarily accompany the pulsatile volume changes peripheral to the orifice meter can be regarded as the volume-elastic (V-E) flow components. These oscillatory components of the inflow pattern therefore represent the changes in the rate of flow to the distensible arterial reservoir through which are mediated the corresponding phasic changes in intravascular volume (volume-elastic volume changes within the bed).

Using the same arterial inflow pattern (fig. 2) the analysis can be extended to the consideration of single points on the inflow curve in relation to simultaneous points on the venous outflow curve. An algebraic summation of the two reveals the contribution made to the inflow curve by the "volume-elastic flux" of blood within the arterial bed. Considering first, point *I* in figure 2 on the ascending systolic flow limb, the distance *GI* represents the rate of inflow recorded at the flow meter; *GH* represents the mean rate of flow through the bed, and *HI* the rate of V-E "flow of accommodation" within the bed. At point *J*, the inflow, mean rate of flow, and outflow are the same, indicating no V-E volume change within the bed. At point *K* the distance *KL* indicates a small rate of inflow. Since *NL* shows the outflow rate to be as large as *HG* (at point *I*) it must be assumed that *NK* represents the rate at which the arterial bed decreases in volume (recoil) to maintain the same rate of outflow. At point *P*, the rate of V-E recoil, *RP* is so great that a backflow of blood progressing at the rate of *PQ* occurs in spite of the constant outflow, *QR*. At point *S*, the rate of recoil, *TS*, is equal to the outflow rate *ST* and therefore the rate of inflow at the meter is equal to zero. Such an analysis should hold for any peripheral

arterial bed where the arterial inflow pattern is known and the venous outflow is essentially nonpulsatile.

It may therefore be stated that: 1. The rate of venous outflow from a peripheral bed, under reasonably constant conditions, is identical in magnitude to the mean rate of arterial flow into the corresponding bed, which value, in turn, determines the *placement* of the flow pattern above the zero flow line. 2. The algebraic difference between the rate of arterial inflow and the rate of venous outflow expresses the rate and direction of "V-E flow," of which the flow pattern *contour* is a continuous graphic representation. The "V-E flow," although recorded as an axial flow, is actually a radial displacement of blood within the vessels. The direction of the radial displacement (centripetal or centrifugal) will depend upon the pressure gradient at the moment. The pressure gradient is, in turn, determined by the character of the applied pressure pulse (central to the flow meter) and the V-E properties of the arterial tree and adjacent extravascular tissues (peripheral to the meter). 3. Whenever the rate of retrograde "V-E flow" (centripetal intravascular recoil) *exceeds* the rate of peripheral outflow, the arterial flow pattern will reveal a backflow component. The magnitude of the backflow will depend upon the duration and magnitude of such an *excess*.

The phasic fluctuations in intravascular volume versus the accompanying phasic fluctuations in applied pressure head constitutes a special type of V-E relationship. Unlike a "static" V-E (SV-E) relationship which exists only under static conditions of equilibrium, the relationship here of a changing volume to a changing pressure exhibits several important differences. The latter may be attributed to the influences of inertia and viscosity which appear only in the "dynamic" state of pressure-flow-volume change, and whose effects will be considered later.

The determination of a V-E curve under static conditions (SV-E curve) which will have reliable significance under normal experimental (dynamic) conditions *in vivo* does not seem possible at present. However, it appears justifiable to evaluate changes in the flow pattern in terms of the probable and logically anticipated changes undergone by the theoretically existent SV-E curve.

Within limits it would be expected that the net effects of vasoconstriction and dilatation will be that of decreasing and increasing respectively the intravascular volume change which will result from a given change in distending pressure. The recorded changes in flow patterns presented elsewhere (18) contribute support to this belief. In the case of vasoconstriction the effect on the SV-E curve will be that of displacing it away from the volume axis, while dilatation, increasing the sensitivity of volume response, shifts the curve toward the volume axis.

As outlined previously, the cyclic volume changes within the arterial bed are manifested in the inflow pattern as the V-E components. It therefore will be expected that vasoconstriction will have the effect of diminishing the magnitude of the V-E flow components (see areas *A*, *B*, *C*, *D*, *E* and *F*, fig. 2) thereby indicating smaller cyclic volume changes within the bed. Similarly, vasodilatation will permit a greater cyclic volume change within the bed and there-

fore V-E flow components of greater magnitude in the flow pattern. The effects of vasomotor drugs upon the flow pattern have been recorded and are presented in a companion paper.

In continuation of the flow pattern analysis, it becomes evident that "static" V-E relationships alone are inadequate for an evaluation of the constantly changing or "dynamic" pressure-flow-volume relationships existing within the period of a single flow cycle. The dynamic factors of inertia and viscosity introduce a considerable variation in the magnitude and timing of the intravascular volume response to applied pressure change; as the result of inertia the volume change may lag behind or possibly overshoot its corresponding change in applied pressure while viscosity imposes a considerable damping and retardation of the volume change.

The magnitude of the combined effects of inertia and viscosity can be evaluated from the same flow and pressure curves reproduced in figure 3. By graphic integration of the velocity curve, F, the corresponding volume pulse curve V has been constructed.[8] The fact that the intravascular volume change is not synchronous with the applied pressure change is revealed by the temporal disagreement between comparable segments of the pressure and volume curves. The relationship of pressure to volume can be better visualized when synchronous points on the two curves are plotted against each other, cf. figure 4. In this form the dynamic V-E relationship appears as a continuous circuitous curve. Parallel to the long axis of the curve and passing through its apices is line S, which has been inserted to represent a conceivable and not improbable general position of the "simultaneously existing static V-E curve."[9]

In relation to the *SV-E* curve, the *DV-E* curve is displaced, first, away from the volume axis during the major pressure rise, and then toward the volume axis

[8] No means are available for determining, in a given bed, the contained volume of blood between the flow meter and the point wherein flow pulsations are essentially nil. While curve P indicates the applied pressure changes which exist above a known diastolic level, curve V will show the intravascular volume changes which are superimposed upon an unknown total intravascular volume. Therefore, a graph of applied pressure versus intravascular volume increment (fig. 4) will represent the "dynamic" relationship of applied pressure (not distending pressure) to volume change without reference to absolute position along the volume axis. It is realized that the applied pressure, which is recorded at the meter, bears no constant relationship to the mean distending pressure throughout the arterial tree and further ignores the presence of those changes which may occur in intracapillary pressure.

[9] The "static V-E curve" referred to here could be experimentally determined only if it were possible to remove suddenly from the arterial bed all dynamic and physiological influences, i.e., flow through the bed with its viscosity and inertia effects, nervous and metabolic influences upon the size and distensibility of the vessels, etc. In this hypothetical state a single static V-E curve could be determined at leisure which would represent the pressure-volume relationship for the same bed, with the same vasomotor and elastic state of the vessels but without the two dynamic influences of viscosity and inertia. The basic, although theoretical, existence of this "static V-E relationship" in a dynamic system is a concept essential to this analysis. However, the precise position and exact slope of its graphic representation (curve S, fig. 4) need not be known for the illustrative and comparative purposes for which it is used here.

as the pressure is falling. Both displacements indicate that the volume change lags behind the pressure change. While it is impossible to identify and separate the inertia and viscosity effects, the latter appear to exert the greater dynamic influence and will be considered in more detail.

As applied pressure and flow velocity rise at the beginning of the cycle (see figs. 3 and 4) the increase of intravascular volume lags behind the rise in pressure. Similarly, during the fall of the major pressure wave the rate at which the volume of the bed decreases is likewise retarded. Since the pulsatile volume

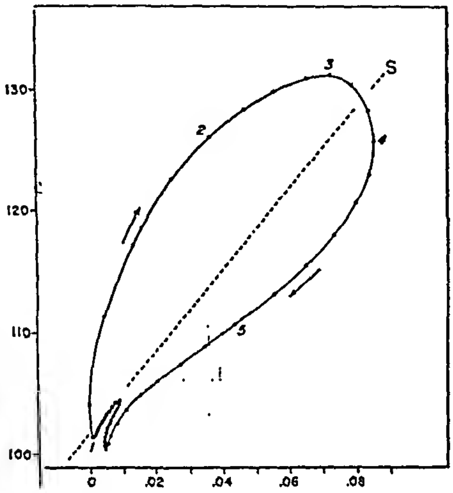

Fig. 4

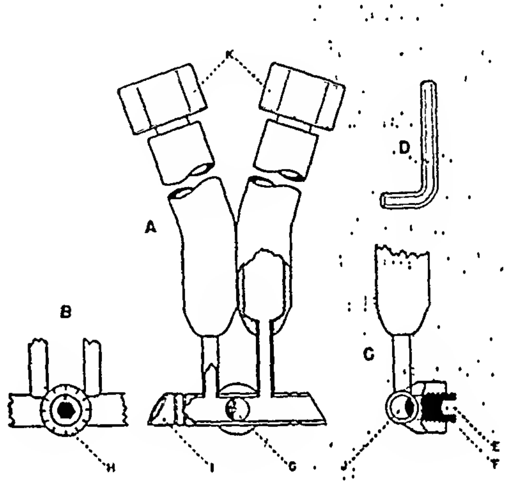

Fig. 5

Fig. 4. Graph showing a continuous plot ("dynamic" V-E curve) of applied pressure versus intravascular volume increment derived from flow curve *F*, figure 3. Ordinate, applied intra-arterial pressure in millimeters Hg. Abscissa, intravascular volume increment in cubic centimeters. Dots on curve demarcate uniform time intervals (1/50 cycle length). Numbered dots indicate time relations of correspondingly numbered intercepts in figure 3. Arrows indicate direction of progression with time. Interrupted line *S*, presumptive trend of "static" V-E curve.

Fig. 5. Semi-diagrammatic sketch of modified orifice type flow meter with readily adjustable orifice. *A*—back, *B*—front, and *C*—side views of meter with cut-away sections showing relations of internal parts. *D*, key wrench for adjusting size of orifice. *E*, key socket. *F*, threaded stud screw with hemispherical end protruding into lumen of tube. (See end view, *G*.) *H*, circular scale for orifice settings. *I*, end, cannula-shaped. *J*, lumen of meter tube. *K*, female connections for attachment to optical differential pressure recorder.

changes are mediated through pulsatile changes in rate of flow to the bed, it is reasonable to believe that the former will be diminished or damped in proportion to the extent to which frictional resistance diminishes or damps the flow of blood to, and exchange of blood within, the bed. The net effect upon the applied pressure-intravascular volume relationship (fig. 4) can be summarized as an inability of the volume change to "follow" the rapid applied pressure change, due to the high viscous damping within the fluid system.

In summarizing to this point the foregoing analysis as applied to the original

flow pattern (fig. 2) or to any other peripheral arterial flow pattern, the following may be said: the flow curve is a record of the direction and velocity with which blood flows by a point in a peripheral artery supplying a given bed. Since the peripheral outflow from the same bed is essentially nonpulsatile, variations in the rate of inflow must reflect the capacity of the arterial tree to distend and recoil during the cyclic rise and fall of the applied pressure pulse. Basically, the theoretical volume which can be accommodated for a given rise in pressure (SV-E relationship) is determined by the existing vasomotor state and elastic properties of the arterial vascular tree. To deliver that theoretical volume throughout the tree in a dynamic system it would be necessary that the inflow acquire a velocity of sufficient magnitude so that the volume to be accommodated is delivered within the period of time during which pressure is rising. In the reverse situation, as the applied pressure is falling, the amount by which the intravascular volume diminished should be compensated by an appropriate and simultaneous decrease in the rate of inflow. However, such theoretically ideal circumstances do not prevail in a dynamic system since fluid friction (viscosity) diminishes greatly the velocity with which blood flow responds to pulsatile changes in applied pressure. Consequently, the intravascular volume *response* lags behind and undoubtedly fails to reach its theoretical value based upon the existing applied pressure. This damping effect is reflected in the inflow pattern as a diminution in the magnitude of deviation from the mean rate of flow and in the rapidity with which flow responds to change in applied perfusing pressure.

Extending further the analysis of flow pattern, a consideration of conditions in the experimental animal reveals that several factors, collectively and interdependently, may determine the *extent* of viscous damping which will exist *at any given moment* in the cycle. Divided into two classes, they are those which affect 1, the velocity of flow, and 2, the intravascular bore (as it determines internal surface area/volume relationship). In class I are *a*, the magnitude of volume flow through the bed (position of pattern above zero flow level), and *b*, rate of change of applied pressure. In class II are *a*, active or passive constriction, and *b*, active or passive dilatation of the arterial vessels. The magnitude of viscous damping will vary in the same direction as do the changes in 1*a*, 1*b*, and 2*a*, and in the reverse direction from those in 2*b*. However, the magnitude and direction of the effects of a single variable are frequently unrecognizable and may be complicated by associated changes in other variables. For instance, decreasing intravascular bore by active vasoconstriction (2*a*) will augment viscous damping by increasing the internal surface area/volume relationship, but under certain circumstances the effect may be partly or completely nullified, or even over-compensated by an accompanying decrease in mean flow (1*a*) and/or an increase in the pressure pulse. The latter will induce a certain degree of passive mechanical dilatation (2*b*) at the higher pressure values. It will elicit not only an absolute increase in intravascular volume *exchange* by virtue of the greater pressure extremes, but also a relative decrease because of the accompanying increase in the rate of change of applied pressure (1*b*). The same considerations may be applied to the oppositely directed changes associated with active dilatation of the arterial vessels.

The interrelationship of the above factors is of such complexity that the effects of a given variable can be evaluated only with respect to the net effect induced by it and other dynamically associated factors (18).

Unfortunately the above analytical approach does not encompass all of the factors which comprise the determinants of coronary flow patterns. The latter are influenced by the effects of cyclic changes in intravascular volume associated with compression and relaxation of extrinsic origin (extravascular support). In addition coronary venous outflow is markedly pulsatile. Consideration of coronary flow patterns will be made in a future communication.

SUMMARY

Flow patterns (and simultaneous intra-arterial pressure curves) have been optically recorded with an improved orifice-type flow meter in the renal, hepatic, superior mesenteric, femoral, axillary, and common carotid arteries of dogs to which has been administered only anesthetic and anticoagulant.

A flow pattern may be characteristically distinctive of a given artery and its bed, but flow patterns in heteronymous arteries are found to exhibit wide variations in magnitude, timing, direction, and rate of flow and in similarity of contour to their respective pressure pulses. Back flow components have been consistently found to exist in the femoral and axillary arterial flow patterns, frequently found in the common carotid patterns, while the renal, hepatic, and superior mesenteric have exhibited only forward flow.

A study has been made revealing the probable determinants of, and their interrelated influences upon, the phasic rate of inflow to a bed (flow pattern) under the above and other physiological conditions.

Although the above analysis does not lend itself to a *quantitative* evaluation of the static and dynamic factors which initiate and moderate the phasic rate of inflow to a bed, it constitutes a basis for a *qualitative* evaluation of differences among, and changes in, flow patterns recorded in the same or different arteries under various physiological conditions.

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A STUDY OF FLOW AND PATTERN RESPONSES IN PERIPHERAL ARTERIES TO THE INJECTION OF VASOMOTOR DRUGS[1, 2]

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In a previous communication (1) there were presented a method of analysis and interpretations of flow patterns recorded in various peripheral arteries of the anesthetized dog. In association with that study flow patterns have been recorded under a great variety of conditions. Some of the more interesting pattern changes have been observed to follow the injection of vasomotor drugs. The phasic flow curves and their interpretations presented here will reveal, in part, those dynamic changes which occur locally as well as those occurring in the general circulatory system.

The preparation used here was similar to that previously described (1) and, in brief, consisted of an anesthetized dog to which antigoagulant had been administered. Phasic arterial pressures and flow rates were recorded simultaneously and continuously by the optical methods of Gregg and co-workers (1, 2, 3, 4, 5).

Transformations in flow pattern and changes in mean rate of blood flow were induced in the renal, superior mesenteric, hepatic, common carotid, axillary and femoral arteries by the injection of vasodilator and vasoconstrictor drugs. The drugs were injected either intravenously or intra-arterially and continuous records were taken. By the latter route local and general systemic effects could be temporarily separated so that the immediate local effects upon flow and pattern could be recorded before the delayed and more general systemic effects appeared. Following the intravenous injection the combined effects of the local and general systemic changes on the magnitude of flow and pattern were observed to appear more nearly simultaneously.

In the interpretation of changes in the flow curves to be presented here the significant considerations are those which have been previously set forth (1): 1, the mean height of the flow pattern above the zero flow level which indicates the magnitude of mean rate of flow through the bed, and 2, the volume of pulsatile deviation from the mean flow line which reflects the dynamic volume-elastic properties of the arterial tree.

In addition, vasomotor changes within a bed can be recognized under certain conditions by correlating, as a simple ratio, the mean flow value with the corresponding mean applied pressure value. However, the application of such an index is limited and should be made with caution. Such an index does not take

[1] The expenses of this investigation were defrayed to a large extent by a grant from the Commonwealth Fund.

[2] Preliminary reports of a part of this work were presented before the American Physiological Society at Chicago, April, 1941, and at Boston, April, 1942.

into account viscosity effects which, in a dynamic system, may give rise to considerable variation in the relationship of mean pressure to mean flow, for which appropriate *in vivo* correction curves are not obtainable. Nevertheless, it is believed that the vasomotor state of a bed may be regarded as having changed when mean flow and mean blood pressure undergo considerable change in opposite directions.

As recorded in the inflow pattern, the pulsatile deviation from the mean rate of flow (dynamic volume-elastic flow components) varies directly with the pulsatile differential pressure at the site of the meter. However, the magnitude of the volume flow represented by these waves is limited by the extent to which fluid friction (viscous damping) retards the rate of intravascular volume exchange (dynamic volume-elastic "flow") of blood. The interpretation of changes in the flow patterns will deal with the interrelationship of the above factors whose respective determinants and mode of operation have been presented in a previous analytical study (1).

A condensed description and interpretation of typical transformations in flow pattern found to occur in various arterial beds following the injection of drugs is presented below. For better evaluation of flow pattern changes the curves have been rectified to a linear scale and the mean flow level indicated. In some instances reproductions of the original flow curves are also included.

Intra-Arterial Drugs. Renal artery—nitroglycerine (fig. 1A). Mean flow first increases and then decreases as the local and more general systemic vascular responses appear, respectively. The dynamic volume-elastic (DV-E) component, while relatively unchanged at 22 seconds, soon becomes larger and later quite small. The appearance of back flow has never been observed in the renal artery following the injection of vasodilator drugs.

The explanation for these flow and pattern changes is reasonably clear. The renal vascular bed obviously dilates initially (since blood pressure falls but mean flow increases). Dilatation possibly continues throughout the period of fall of blood pressure but is difficult to establish with certainty since both blood pressure and blood flow change in the same direction. Since at the time of maximal mean flow (22 sec.) the magnitude of the DV-E flow components is not appreciably altered, it is suggested that the accompanying increase in rate of flow has imposed a relatively greater viscous retardation of DV-E flow (intravascular volume exchange). The effect of a diminished rate of flow in decreasing viscous damping is suggested in the record taken 54 seconds after injection when the DV-E components are much larger than the control in spite of a smaller pressure pulse.

Renal artery—suprarenin (fig. 1B). Mean flow is promptly reduced, almost to zero. The DV-E components become very small in spite of an increased pulse pressure and the flow pattern is grossly changed and transformed into a number of small, sharply angular oscillations, some of which descend below the zero flow line, thereby constituting periods of back flow. The direction of each flow fluctuation is in phase with a corresponding rapid change in pressure gradient. (Note transitional stages in 1–2 sec. record.)

It is evident that local vasoconstriction is marked. A reduction in DV-E

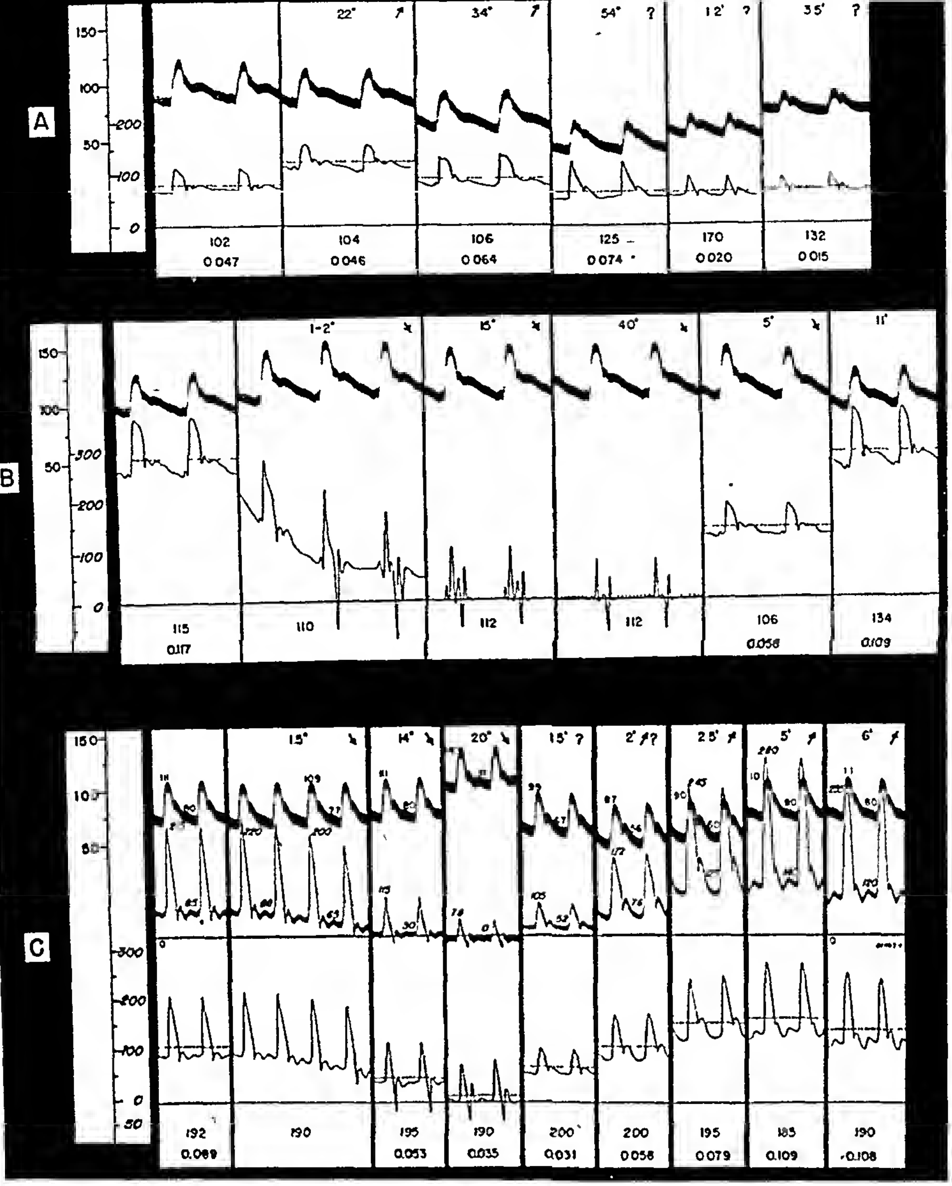

Fig. 1 A. Reproductions of a series of original pressure (upper) curves and rectified reconstructions of original velocity flow (lower) curves recorded in the renal artery following the intra-arterial injection of nitroglycerine (1.2 mgm.). Dog weight 15.4 kgm. Ordinate scales—pressure in millimeters of mercury on left. Flow in cubic centimeters per minute on right. Continuous horizontal line indicates zero flow level. Interrupted line indicates mean flow level for each record segment. Numbers on each record segment, reading consecutively from top to bottom are: time elapsed following drug injection, heart rate, and volume-elastic flow component (increment or decrement) in cubic centimeters per cycle. Change in vasomotor state of bed with reference to control state is indicated in right upper corner of record segment by: ↗ -dilatation, ↘ -constriction, ? - not known.

Fig. 1 B. Renal arterial pressure (original) and flow (rectified) curves showing serial effects of intra-arterial injection of suprarenin (0.05 mgm.). Dog weight 15.4 kgm. Order of curves, notations, and ordinate scales same as figure 1 A.

Fig. 1 C. Reproductions of original pressure (upper) and flow (middle) curves with rectified flow curve reconstructions (lower) recorded in the hepatic artery following the intra-arterial injection of suprarenin (0.10 mgm.). Dog weight 12.7 kgm. Numbers on pressure curves indicate systolic and diastolic pressure values in millimeters of mercury. Slanting numerals on original flow curves indicate late diastolic and maximal systolic rates of flow in cubic centimeters per minute. Other notations and ordinate scales same as figure 1 A.

components exists in spite of an increased pulse pressure and lowered mean flow, and occurs, presumably, as a result of diminished elastic capacity of the constricted vessels and the augmented viscous retardation imposed by the associated reduction in bore.

Hepatic artery—suprarenin (fig. 1C). The changes in flow pattern, the appearance of back flow, and the reduction in DV-E component during vasoconstriction are quite similar to those found in the renal artery and are to be ascribed to the same influences. On the basis of the criteria set forth above, the vasomotor change in the bed at 1.5 and 2 minutes is that of dilatation with reference to the 20 second record but its state cannot be determined with respect to the control state, since both blood pressure and mean flow have changed in the same direction. Later, during the recovery phase, marked vasodilatation appears and is accompanied by an increased DV-E component. The most important determinant of the augmented DV-E fraction is presumably the ability of the dilated vascular bed to undergo greater pulsatile volume changes. The secondary dilatation may also appear in the renal artery but does not invariably occur in either artery.

Common carotid artery—nitroglycerine (fig. 2A). Mean flow first increases and then drops (as blood pressure falls) toward the control value. The pattern most nearly resembles the contour of its pressure pulse at the maximal rate of flow, this resemblance diminishing as blood pressure and flow decline. The DV-E component, relatively unchanged as mean flow increases, becomes very large as blood pressure falls and a small back flow appears in early diastole.

Local dilatation is immediate and persists throughout the 2 minutes of records. The DV-E changes have a similar origin to that described for the renal artery following injection of the same drug. The appearance of back flow is obviously associated with the increased amplitude of the DV-E component occurring in conjunction with a lowering of the mean flow level.

Similar but more prolonged effects are obtained following the injection of aminophylline, with the exception of the fact that the fall in blood pressure and mean rate of flow is not as great and no back flow appears (records not shown).

Common carotid artery—suprarenin (fig. 2B). Mean flow is progressively reduced and remains low despite the subsequent large elevation of blood pressure. The flow pattern contour undergoes only minor changes initially but, as blood pressure and pulse pressure increase, the flow pulse amplitude and DV-E component increase greatly and several back flow components of considerable, though varying, amplitude and magnitude appear during diastole. The appearance of back flow with diminished mean rate of flow has been previously observed by Katz and Kolin (6).

Marked vasoconstriction is obviously present. The increase in the DV-E component, in contrast to that following the injection of the same drug in the renal and hepatic arteries, is probably related to the dominant influences of the markedly increased pulse pressure and decreased mean flow over the combined damping effects of reduced vascular bore and increased rate of DV-E flow.

Comparable results are obtained with pitressin except that, since the pressure

pulse is only mildly elevated, the flow pulse amplitude and DV-E fraction are *only moderately increased (records not shown).*

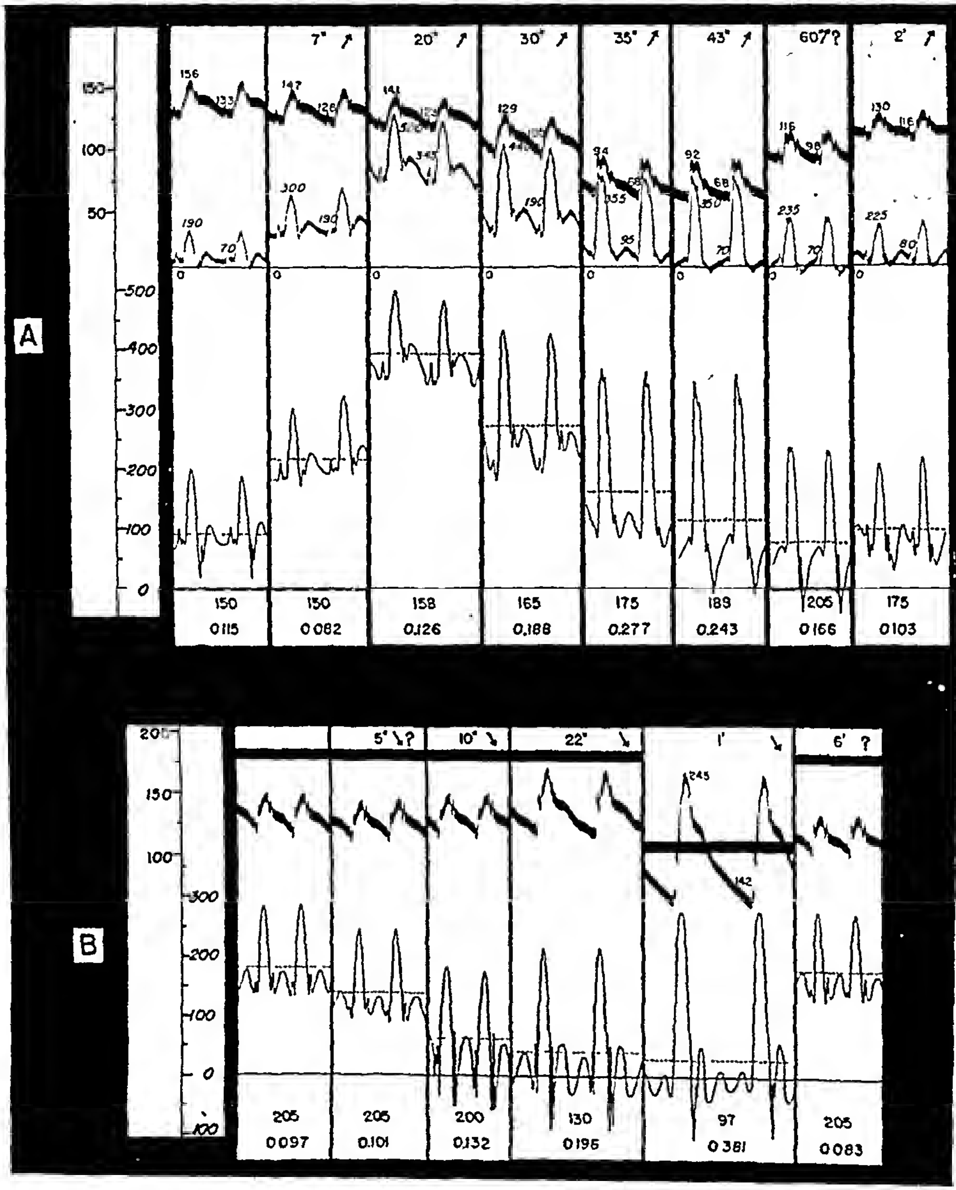

Fig. 2 A. Original pressure and flow curves with rectified flow curve reconstructions recorded in common carotid artery following intra-arterial injection of nitroglycerine (0.3 mgm.). Dog weight 12.6 kgm. Order of curves, notations, and ordinate scales same as figure 1 C.

Fig. 2 B. Common carotid arterial pressure (original) and flow (rectified) curves recorded following intra-arterial injection of suprarenin (0.1 mgm.). Dog weight 13.4 kgm. Order of curves, notations, and ordinate scales same as figure 1 A.

Femoral artery —nitroglycerine (fig. 3A). As with responses to vasodilator drugs in other arteries, mean flow at first increases and then decreases. Both

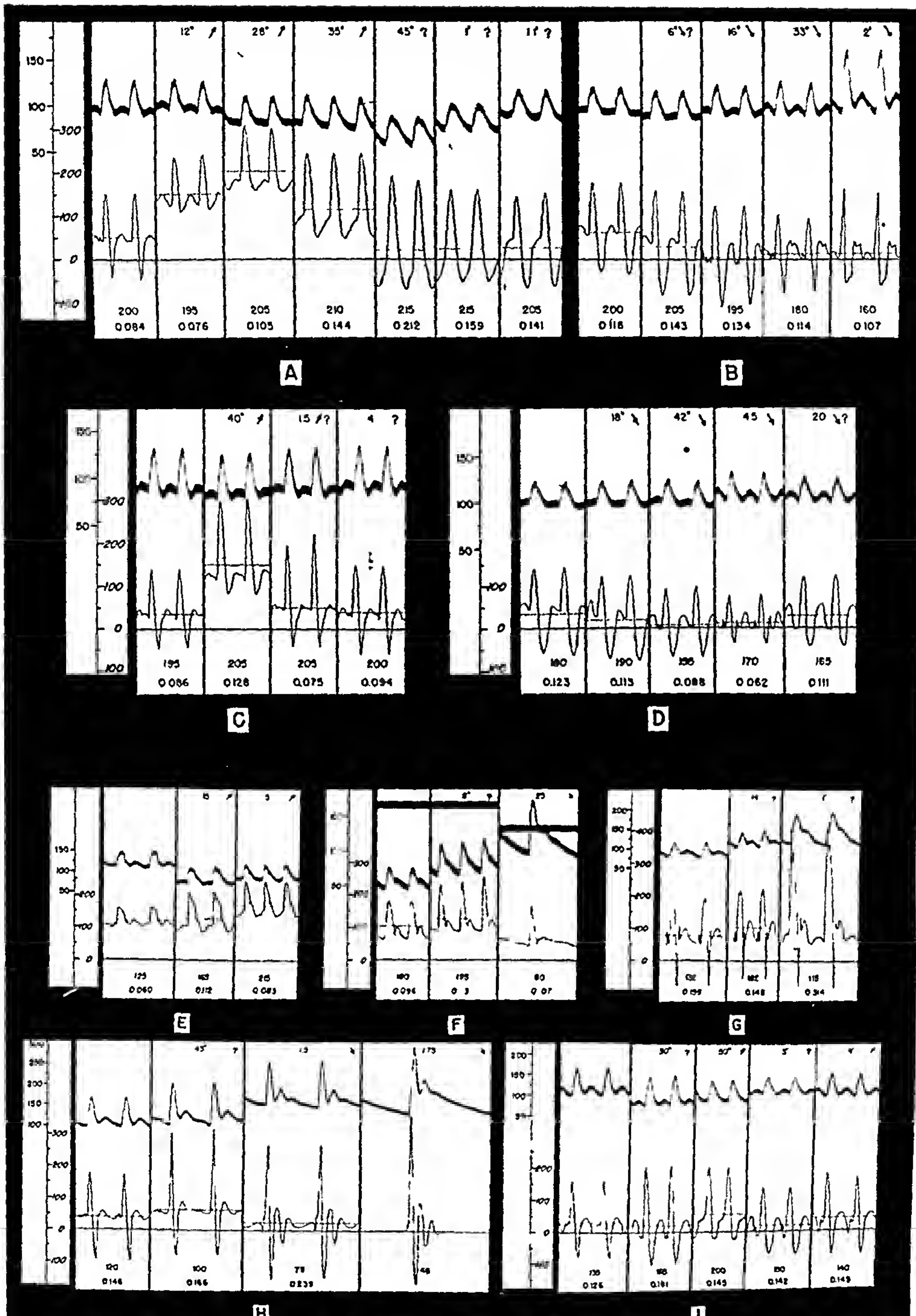

Fig. 3 A through D. Femoral arterial pressure (original) and flow (rectified) curves recorded following the intra-arterial injections of vasodilator and constrictor drugs. Part A—nitroglycerine (0.3 mgm.). Dog weight 11.2 kgm. Part B—suprarenin (0.1 mgm.). Dog weight 13.4 kgm. Part C—aminophylline (24 mgm.). Dog weight 13.5 kgm. Part D—pitressin (12 units). Dog weight 13.4 kgm. Order of curves, notations and ordinate scales same as figure 1 A.

Fig. 3 E through I. Reproductions of pressure curves (original) and flow patterns (rectified) recorded in different arteries following the intravenous injections (via external jugular) of vasodilator and vasoconstrictor drugs. Part E—renal artery, theamin (250 mgm.). Dog weight 14.0 kgm. Part F—hepatic artery, suprarenin (0.2 mgm.). Dog weight 12.7 kgm. Part G—common carotid artery, suprarenin (0.06 mgm.). Dog weight 10.4 kgm. Part H—femoral artery, suprarenin (0.07 mgm.). Dog weight 11.4 kgm. Part I—femoral artery, nitroglycerine (0.3 mgm.). Dog weight 11.4 kgm.

systolic and diastolic portions of the flow pattern become full, more rounded, and the "normally" existent back flow disappears with the elevation of mean flow. As mean flow (and blood pressure) drop the DV-E component increases markedly and back flow of considerable magnitude reappears, occupying most of diastole. The changes in pattern and the mechanisms responsible for them are similar to those described for the effects of nitroglycerine in the renal and common carotid arteries.

Injection of aminophylline (fig. 3C) produces changes which are smaller but quite similar to those occurring initially after injection of nitroglycerine, i.e., mean flow increases, obvious dilatation occurs, and the back flow disappears, but the flow pattern and the DV-E fraction are not greatly altered.

Femoral artery—suprarenin (fig. 3B). The mean flow rapidly reaches a low level and remains low despite the subsequent marked augmentation in blood pressure. The flow pattern is only moderately altered in contour but the back flow is of greater amplitude and volume. The DV-E fraction at first increases mildly, but later, at 2 minutes, returns to the control value despite a large pulse pressure. This response contrasts with that observed in the common carotid but compares with the effects observed in the hepatic and renal arteries.

It is evident that relatively marked vasoconstriction appears and persists. Again the relatively small change in the DV-E component is regarded as evidence of a close balance among the effects of increased pulse pressure, decreased mean flow, altered distensibility of the vessels and a greater viscous damping because of their constricted state.

Similar changes in pattern follow the injection of pitressin (fig. 3D) except that a considerable reduction in the DV-E component occurs. This is undoubtedly associated with the failure of the pulse pressure to become augmented sufficiently to offset the damping effects of the same factors as mentioned above.

Correlation of Effects of Drugs Injected Intra-arterially. As anticipated, so-called vasoconstrictor and vasodilator drugs given intra-arterially have caused, in the local bed, vasoconstriction and vasodilatation respectively, i.e., the mean flow has decreased at a higher blood pressure, or increased at a lower blood pressure. However, in some of the above records the systemic effects of the drug have so affected the applied perfusing pressure that these criteria alone are not applicable to reveal changes in the vasomotor state of the bed. Such a shortcoming is particularly evident when interpreting changes following the intravenous injection of drugs.

Certain generalizations can be made regarding the transformations in flow patterns recorded in various peripheral arteries. The existence of a V-E flow component is independent of the mean rate of flow through the bed. Its magnitude may be influenced by variations in the mean rate of flow, but as Hewlett and Van Zwaluwenburg (7) have pointed out, directional changes in either may occur independently of the other. In the early stages following the intra-arterial injection of vasodilator drugs, before the systemic effects appear, the arterial pattern acquires a contour which resembles more nearly that of its corresponding pressure pulse. For a given artery, the similarity of pressure and flow patterns is frequently observed to be greatest when the mean rate of flow is high and

viscous damping of the volume-elastic exchange of blood is thereby presumably increased. Conversely, the similarity is usually least when mean flow is low and the volume-elastic exchange is less damped.

In comparing the drug effects observed in different arteries it must be kept in mind that all beds do not possess the same sensitivity of vasomotor response. In addition, the total volume of the vascular bed will vary in different organs and extremities as will the distribution of that volume among vessels, such as the arterioles, which are relatively more responsive to vasomotor drugs and those vessels, such as the large arteries, which are less responsive. A comparison of the flow patterns obtained in the renal and common carotid arteries following the intra-arterial injection of suprarenin (fig. 1B, fig. 2B) illustrates the inherently different responses in the two beds. In other experiments, renal inflow immediately and rapidly fell to a very low level following a very small injection (of the order of 0.0001 mgm.), while a much larger dose in the common carotid (approximately 0.1 mgm.) was much less prompt and effective in its local constrictive action (records not shown). The marked decrease in the DV-E component observed in the renal, in contrast to the large increase in the common carotid, is undoubtedly associated with the smaller accommodative capacity for volume-elastic exchange in the former. The short length of the renal artery and small total arterial bed volume with a relatively large proportion of arterioles in the kidney constitute a comparatively small reservoir which, in addition, is capable of inducing considerable viscous damping (of flow fluctuations). On the other hand, the common carotid possesses a relatively long system of large arterial branches, a large vascular bed and, presumably, a smaller proportionate volume of arterioles. Thus the effects of arteriolar constriction upon the DV-E component may be easily lost in, and overpowered by, the influence of even a small systemic augmentation of pulse pressure upon the more elastic and accommodative large arterial vessels which impose only a relatively small viscous limitation upon DV-E flow fluctuations and undergo comparatively smaller degrees of constriction. The pattern responses observed in the other arteries appear to fall between the above extremes and vary in relation to the physiological sensitivity and anatomical distribution of the vascular components.

Similarly, the effects of dilator drugs upon the arterial inflow patterns will depend upon the preëxisting vasomotor state of the bed and the same physiological and anatomical factors as those mentioned above.

It is interesting to note that the injection of *either* constrictors or dilators may be associated with the appearance or augmentation of back flow in many arteries. Its existence in any artery may be ascribed to the presence of a single set of conditions, irrespective of the artery, organ, member, drug, or type of response involved. Back flow will be recorded at the site of the meter whenever the applied perfusing pressure on the central side of the meter is less than the pressure produced peripheral to the meter as the result of passive elastic recoil from the arterial tree and surrounding tissue (and the active extrinsically applied pressure in the case of the coronary arteries). Thus the greater the volume-elastic recoil component and the smaller the mean differential pressure (mean rate of flow)

at the site of the meter, the greater will be the back flow component. The rapid back flow and forward flow oscillations recorded in arteries supplying constricted beds, in contrast to the slower waves which accompany the effects of dilators, presumably are related to the higher natural frequency of the arterial bed in the constricted state.

Intravenous Drugs. An intra-arterial injection permits the transient separation of the local from the combined local and general systemic effects of a drug. An intravenous injection (via the external jugular vein) is usually followed by a variable and unpredictable admixture in time of appearance and magnitude of the two responses. Hence, the resultant changes in mean flow and flow pattern very frequently differ from those found to occur after intra-arterial injection. In many instances the vasomotor state of the bed can be established with certainty and the qualitative, if not the quantitative, change in the state is comparable to that obtained following the administration of the same drug intra-arterially. In others the early and/or intermediate (or even late) changes in the vasomotor state of the bed are not determinable from flow and pressure data.

In figure 3, E through I, are presented several series of curves which show the variability in sequence and magnitude of the flow (and blood pressure) response obtained following the intravenous injection of drugs.

In the renal artery (fig. 3E) the response to theamin can be readily interpreted and is similar to that obtained following intra-arterial injection of aminophylline (records not shown).

The intravenous administration of suprarenin causes, in the hepatic and femoral arteries (figs. 3F, H), a rise followed by a fall in mean rate of flow below the control level while blood pressure progressively rises. The later records of flow in both arteries show definite constriction but the rise in mean flow in the early records is presumably a result of an augmented blood pressure which offsets the effect of any possibly coexistent beginning vasoconstriction within the local bed.

The response in the common carotid to intravenous suprarenin (fig. 3G) is a progressive rise in mean flow and blood pressure. It is probable that the elevation in mean flow is associated with the greater driving force of an elevated systemic blood pressure which outweighs the effect of a mild constriction of the bed. However, with the recorded data alone, the possibility cannot be excluded that the bed is either unaffected by the drug, passively dilated, or even actively dilated.

A variance in dominance of peripheral and general systemic drug effects is apparent in the femoral flow curves following nitroglycerine injection (fig. 3I). With the exception of the 50 second and 9 minute records (where dilatation is present relative to the control state), mean flow and blood pressure directional changes are such that the vasomotor state of the bed cannot be identified.

SUMMARY

Optically recorded flow patterns (with an improved orifice type meter) together with the coexisting pressure pulses in the superior mesenteric, hepatic, renal,

common carotid, axillary, and femoral arteries of anesthetized dogs are presented. The effects of intra-arterial and intravenous administration of vasodilator and constrictor drugs on the flow and pressure curves are shown. Following the system of analysis previously described (1), several series of curves have been examined with reference to changes in 1, mean flow; 2, relationship of mean flow to mean pressure; 3, similarity of contour between patterns and pressure pulses; 4, volume of pulsatile deviation from the mean rate of flow (dynamic volume-elastic properties of the vascular tree), and 5, the vasomotor state of the bed (dilatation or constriction).

The generally accepted vasomotor responses to so-called dilator and constrictor drugs given intra-arterially were observed. However, in some records, and especially in those obtained following intravenous drug administration, changes in the vasomotor state of the bed could not be determined because of the overlapping of central and peripheral drug effects.

The dynamic volume-elastic component is reduced, except in the common carotid artery, by vasoconstrictor drugs given intra-arterially, while vasodilators usually increase this fraction during most of the period of drug action.

The injection of constrictor drugs, either intravenously or intra-arterially, may cause the appearance or augmentation of back flow in the patterns of all peripheral arteries so far studied. Dilators may introduce backflow in the common carotid pattern and greatly augment that preëxisting in the common carotid, axillary, and femoral arterial patterns.

The interrelationship of the determinants associated with vasomotor drug effects is considered and discussed.

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EFFECT OF pH AND CERTAIN ELECTROLYTES ON THE METABOLISM OF EJACULATED SPERMATOZOA[1]

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Within recent years there has been an increased interest in the study of the metabolism of spermatozoa. Variations in methods used in collection of semen, preparation of sperm suspensions and manometric measurements, and variations in composition of the suspension medium used in different laboratories have yielded apparently conflicting results. In order to establish optimum conditions for studying the metabolism of spermatozoa a study was undertaken to determine the influence of the ionic composition of the medium on respiration, glycolysis and motility.

METHODS. The mammalian spermatozoa used in these experiments were from normal ejaculates collected by means of an artificial vagina. The cock semen was collected from White Leghorn cocks by Dr. W. W. Cravens using a modification of the Burrows and Quinn (1) method of collection. Aliquots of the semen were accurately measured into centrifuge tubes and diluted with 1 to 3 volumes of the medium in which the sperm were to be suspended for experimentation. After centrifuging for 5 to 10 minutes the supernatant fluid was discarded. The desired amount of medium was added to each tube and the spermatozoa were suspended by drawing the medium into a 1 cc. serological pipette (at least 1 mm. inside diameter at the tip) and gently blowing it part way out. If the tip of the pipette is kept below the surface of the medium and a few millimeters above the centrifuged spermatozoa the pumping action will suspend the spermatozoa evenly throughout the medium. This process eliminates the mechanical damage, clumps or foam which may occur when stirring or shaking is employed.

As a medium for suspending the spermatozoa, calcium-free Ringer-phosphate was employed. This was prepared (with water re-distilled from glass) according to Krebs (2) except for the omission of $CaCl_2$ and was adjusted to the desired pH (glass electrode) by the addition of either 0.1 N HCl or 0.1 N NaOH. Media for studying the effects of various ions on sperm metabolism were prepared by omitting the ion in question from, or by adding the ion (in 0.154 M solution) to the Ringer-phosphate solution.

Oxygen consumption of the sperm suspension (always 3 cc. final volume) was measured at 37° in air, using either the Warburg or Barcroft apparatus. The

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central cup of each flask contained 0.2 ml. of 20 per cent KOH and a folded strip of porous filter paper to absorb CO_2. To prevent creeping of the alkali, anhydrous lanolin was spread over the rim of the central cups. The manometers were shaken at a rate of 110 oscillations per minute and a 10 to 15 minute equilibration period was allowed before the stopcocks were closed.

Lactic acid was determined by the method of Barker and Summerson (3). Motility was observed on a specimen from each flask after the experimental period as described previously (4). Where metabolites (glucose, lactate, pyruvate) were added, the final concentration in the flask was always 0.02 M.

The results are expressed according to the method of Redenz (5). Z_{O_2} = c.mm $O_2/10^8$ cells/hr. Z_L = lactic acid produced (equivalent to CO_2)/10^8 cells/hr.[2] Sperm concentrations were determined by counting in a hemacytometer. For purposes of comparison of ejaculated bull spermatozoa with other tissues the Z_{O_2} equals $2.5 \times Q_{O_2}$ (7).

We prefer to separate the spermatozoa from the seminal fluid and to study their metabolism in a medium of known composition. This eliminates the influence of metabolites in the seminal fluid as well as the endogenous respiration of the fluid itself (8). The errors involved in measuring oxygen consumption from a medium containing bicarbonate, as in the case of seminal fluid, have been discussed by MacLeod (9). The endogenous respiration has been shown by Zeller (10) to be in part due to the oxidative deamination of spermine by the enzyme diamine oxidase, and it seems likely that the oxidation of ascorbic acid may also be of importance. Bull semen contains on the average 6 mgm. ascorbic acid per 100 ml. (11) and we have found added crystalline ascorbic acid to be oxidized by seminal fluid.

Results. The effect of pH of the medium on the endogenous respiration of washed bull spermatozoa was studied over a range of pH = 6.6 to 7.5. Maximum respiration was obtained at pH = 6.9 to 7.03. With media of lower pH there was a gradual drop in respiration until at pH = 6.68 the oxygen consumption was 85 per cent of the maximum. A more rapid drop occurred on the alkaline side of optimum and at pH = 7.2 respiration was only 75 per cent of the maximum. More alkaline conditions up to pH = 7.5 did not further depress respiration. Motility was not greatly affected by variations in pH from 6.8 to 7.5 but dropped off rapidly below pH = 6.7. Although the magnitude of the respiration of spermatozoa from various bulls may vary considerably the curves plotting the effect of pH on per cent of maximum respiration were remarkably similar.

In the case of rabbit spermatozoa the optimum pH for both respiration and motility was at 6.8. This optimum was in a much narrower range than in the case of bull spermatozoa and the motility was far more sensitive to changes in pH. Oxygen consumption decreased on both sides of the optimum (75 per cent of maximum at pH = 6.0 and 7.7) but depression of motility was relatively greater on the acid side.

[2] Henle and Zittle (6) employ the symbol Z_G to designate glycolysis as measured by CO_2 replacement. Since the values in this paper are calculated from lactic acid determined by a specific chemical method the subscript L is used instead.

The optimum pH for endogenous respiration of cock spermatozoa was 7.25. Motility was equally good over the pH range studied (6.6 to 7.7) but we have found that the optimum pH for maintenance of fertility of cock sperm is near the optimum for respiration.[3]

As reported previously (7), the Z_{O_2} remains constant for sperm concentrations of 100 million to 1 billion spermatozoa in 3 cc. suspension. Henle and Zittle (6) also obtained linear respiration in this range of sperm concentration when epidid ymal secretion was added to epididymal spermatozoa.

Using suitable sperm concentrations and media of optimum pH for the species the Z_{O_2} values shown in table 1 were obtained.

Table 2 shows the effect of various ions and combinations of ions on the metabolism of bovine spermatozoa. The experiments cited are typical of two to six separate studies of the experimental variations. In each experiment calcium-free Ringer-phosphate was used as a reference for comparison.

TABLE 1

Endogenous respiration of ejaculated spermatozoa

SOURCE	NO. OF EXPTS.	Z_{O_2} (1ST HR.)	
		Range	Ave.
Bull	19	16.1–29.8	21.4
Cock	3	6.0– 7.5	6.9
Rabbit	12	7.0–16.1	10.9
Ram	4*	20.0–27.0	22.4

* pH of medium = 7.25.

All values shown were from experiments in which measurement of respiration was begun within 2 hours of ejaculation. Effect of "age" of bull spermatozoa on the oxygen consumption was studied previously (7).

Respiration was fairly good in physiological saline (expts. 1, 2, and 3) but lactic acid production was not as great as in Ringer-phosphate (expts. 1, 2, and 3). This difference was slightly greater for $Z_L^{N_2}$ values (anaerobic glycolysis). The initial motility in physiological saline was excellent but it was not maintained as well as in Ringer-phosphate Varying the phosphate level from 0 to 0.03 molar did not appreciably affect respiration but small amounts of phosphate greatly improved glycolysis in a 0.9 per cent saline medium (expt. 1). Both respiration and glycolysis were depressed in M/10 phosphate buffer (expt. 1). Addition of chloride ion improved respiration but affected glycolysis adversely indicating that perhaps osmotic relationships rather than lack of chloride ions were responsible for the inferior performance in plain phosphate buffer. Motility in Ringer-phosphate was always superior to that in M/10 phosphate buffer.

Omitting Mg^{++} from Krebs' Ringer-phosphate depressed respiration slightly. Omission of Mg^{++} depressed glycolysis but the magnitude of the depression was not consistent. Motility was usually better when the medium contained Mg^{++}.

[3] Halpin, J., C. E. Holmes, W. W. Cravens, P. H. Phillips and H. A. Lardy. Unpublished data.

Although Mg^{++} is essential for the oxidation of pyruvate by muscle (12) and brain (13), it did not increase the respiration of bull spermatozoa in the presence of pyruvate.

The addition of Mn^{++} decreased motility and anaerobic glycolysis as well as respiration and aerobic glycolysis (expt. 3).

As shown in table 3 calcium depressed both respiration and glycolysis (expt. 4) and was very detrimental to motility of bull spermatozoa. Citrate was added to one flask in experiment 5 to see if binding the Ca^{++} carried to the medium by the sperm cells would influence the respiration. Since spermatozoa do not con-

TABLE 2

Typical examples of the effect of various ions on the metabolism of bull spermatozoa

EXPERIMENT NO.	SPERM COUNT PER FLASK	MEDIUM pH = 7.0	MOLARITY OF				Z_{O_2}			Z_L^{air}
			PO_4	K^+	Mg^+	Mn^{++}	Endogenous	Glucose	Lactate	
1	$\times 10^8$ 7.2	Ringer-phosphate	0.016	0.005	0.0012		12.0	8.4	13.5	18.3
		0.9% NaCl	0.003 to 0.03	0.001 to 0.003			11.9 12.2 ±0.5	9.4	16.7	5.7 16.0
		NaK-phosphate	0.100 0.066*	0.040 0.026			6.5	5.3 7.1	5.2	11.1 8.4
2	5.6	Ringer-phosphate	0.016 0.016	0.005 0.005	0.0012		22.3 18.6	19.5 15.7	19.8 16.1	35.1 26.3
		0.9% NaCl					14.3	15.2		25.7
3	8.2	Ringer-phosphate	0.016 0.016	0.005 0.005	0.0012 0.0012	 0.0011	16.8 15.1	17.2 14.4	16.1	19.8 16.9
		0.9% NaCl					18.4	13.5		9.4

* One cubic centimeter of M/10 phosphate buffer replaced by 0.9 per cent NaCl solution.

tain citric dehydrogenase (14–4) an increased oxygen uptake due to its oxidation would not be expected. It was found (confirmed in several other experiments) that citrate decreased respiration but *improved motility under aerobic conditions.* In experiment 6 an attempt was made to demonstrate the classical $Ca^{++} - K^+$ antagonism. In this experiment where the sperm concentration was high, the effect of Ca^{++} on respiration was not apparent but motility was greatly depressed. The effect of K^+ likewise was not appreciable in experiment 6 although it was clearly beneficial in experiment 7 and others. Experiment 6 was repeated with ram spermatozoa at pH = 7.25 and although $Ca^{++} - K^+$ antagonism was

not demonstrated, Ca^{++} had a slight detrimental effect on respiration at all levels of added K^+. A definite stimulation by K^+ of both respiration and glycolysis was obtained in experiment 7 where the spermatozoa were washed twice in K^+-free Ringer-phosphate and in other experiments where the spermatozoa were stored in the K^+-free medium for 30 minutes and then taken up in fresh media. The effect of K^+ on anaerobic glycolysis was also determined. In one experiment where the higher level of K^+ only slightly increased the oxygen consumption it more than doubled anaerobic glycolysis. In other experiments anaerobic

TABLE 3

Typical effects on bull spermatozoa of varying the K^+ and Ca^{++} concentration of the suspension medium

EXPERIMENT NO.	SPERM COUNT PER FLASK	MOLARITY OF K^+	MOLARITY OF Ca^{++}	Z_{O_2} Endog.	Z_{O_2} Glucose	Z_L^{air}
	$\times 10^8$					
4	6.3	0.005		24.8	24.1	32.7
		0.005	0.0018	18.7	13.6	29.4
		0.005	0.0037	13.3	12.3	23.8
5	7.2	0.003		17.0	11.9	
		0.003	0.0037	6.6		
		0.003*		11.5		
6	13.2			13.6		
			0.0018	13.9		
		0.006		14.4		
		0.006	0.0018	15.5		
		0.012		14.9		
		0.012	0.0018	13.7		
7†	3.7	0.000			15.1	23.0
		0.005			18.5	35.1
		0.044			22.8	33.1

Ringer-Phosphate medium used in all experiments.

* Citrate added to a concentration of 0.02M.

† Spermatozoa washed twice with K^- -free Ringer-phosphate before making up the final suspension.

glycolysis was slightly greater when K^+ was present but the stimulation was not as great as under aerobic conditions. A potassium concentration of at least 0.005 M appeared to be essential for the best motility. Further improvement in motility with higher concentrations of K^+ was not discernible.

Discussion. The calcium-free Ringer-phosphate solution which has proved so suitable for studying the metabolism of surviving tissues is likewise suitable for studying the metabolism of ejaculated spermatozoa when precautions are taken to have the pH optimum for the species. Among the ions studied, phosphate, potassium and magnesium were of importance for the maintenance of

glycolysis, respiration and motility. The observation that phosphate was apparently more important for glycolysis than for respiration might indicate that an exogenous source of phosphate is essential for glycolysis. The sperm itself must originally contain sufficient phosphate for its energy coupling mechanism since respiration and initial motility were excellent in 0.9 per cent saline. A more plausible explanation for the effect of phosphate is its buffering action. In the absence of buffer salts lactic acid produced by the spermatozoa rapidly brings the medium below pH = 6 where survival is greatly impaired.

Magnesium benefited motility and usually increased respiration but had no consistent effect on glycolysis. Potassium stimulated while manganese and especially calcium definitely inhibited all three phenomena.

SUMMARY

From this study of the metabolism of ejaculated spermatozoa in Ringer-phosphate media the following conclusions may be drawn.

1. The optimum pH values for the endogenous respiration of bull, cock and rabbit spermatozoa were 6.9 to 7.0, 7.25 and 6.8 respectively.
2. At the optimum pH for the species the endogenous respiration (measurement begun within 2 hrs. of ejaculation) of bull, cock, rabbit and ram spermatozoa was: Z_{O_2} = 21; 7; 11 and 22 respectively.
3. Varying the phosphate concentration of the medium from 0 to 0.03 M did not greatly affect respiration of bovine spermatozoa but glycolysis, and motility in the presence of glucose, were greatly depressed in the absence of phosphate.
4. The omission of Mg^{++} from the medium depressed respiration, glycolysis, and motility in most specimens but the effect on glycolysis was not consistent.
5. Mn^{++} and especially Ca^{++} affected motility, glycolysis and respiration adversely.
6. At least 0.005M K^+ was necessary for maintenance of optimum motility while this and even higher concentrations stimulated respiration and glycolysis.

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BLOOD KETONE BODIES IN RELATION TO CARBOHYDRATE METABOLISM IN MUSCULAR EXERCISE[1]

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An important problem in the physiology of muscular exercise is the extent and manner of utilization of fat by muscles. Present evidence points to the absence of any direct rôle of fat as a muscular fuel, with the probability of its indirect contribution, possibly through the medium of ketone bodies (1, 2). Ketone bodies are removed from a perfusate by isolated diabetic muscles more rapidly if the muscles are stimulated than when they are at rest (3, 4). The situation in the intact organism somewhat obscures this change but the conflicting results (5, 6, 7) are understandable if one considers the production of ketone bodies by the liver to be increased by exercise (8). Heavy exercise over short periods of time has been shown in both rats and men to produce a drop in ketone bodies with a subsequent rise (9, 10). The evidence for the fall in ketone bodies is derived largely from one experiment in which the subject ran at 10 miles per hour for 20 minutes (11). It therefore appears desirable to place on record the results of further human experiments done in this laboratory as well as guinea pig studies in which the blood ketones were followed along with changes in carbohydrate metabolism.

METHODS. The human experiments were done on two male subjects operating a bicycle ergometer[2] against a resistance which could be varied by weights altering the tension of a brake band. The speed was kept constant by a speedometer wired in an electric bell circuit to signal if the subject was deviating from the prescribed speed. Experiments were conducted with the two subjects on a ketogenic diet when definite ketonuria was present, and on a regular balanced diet. Work was done at various measured rates of working. In some experiments work was repeated every two hours for three trials. Blood samples were taken before and after, and sometimes during the work period, and at intervals after the last trial.

The guinea pigs were run on a motor driven treadmill at 950 yards per hour. Some were sacrificed before fatigue, others at fatigue, and others after periods of recovery. The fatigue point was determined by refusal of the animal to run on stimulation by electric shock. Both fed animals and animals in ketosis from fasting for 24 hours were run. Control animals were sacrificed without running. Blood samples were taken as well as samples of muscle and liver, the latter by quickly freezing the tissues in CO_2 ice and ether.

[1] Assisted by a grant administered by the National Research Council of Canada.

[2] Appreciation is due to the Department of Physical Education, McGill, for the loan of the bicycle ergometer and to the Department of Mechanical Engineering for effecting certain alterations in this apparatus.

Analyses were made as follows: blood ketone bodies from the mercury content in Deniges' precipitate (12) expressed in terms of acetone (conversion factor 0.0633); blood pyruvate in the tungstic acid filtrate after stabilization with iodoacetate as the dinitrophenylhydrazone (13, 14); blood hemoglobin with the Evelyn photocolorimeter; lactic acid in blood and muscles by a modified Miller and Munz technique (15) after extraction of the muscles with trichloracetic acid followed by treatment with copper sulphate and calcium hydroxide; muscle and liver glycogen by the modified Pflüger method (16); sugar in blood and in the glycogen hydrolysates by Somogyi's modification of the Shaffer-Hartman method (17). With regard to the method for ketone bodies used, it was established that the amounts of lactate in the blood did not interfere.

RESULTS. *Human experiments.* On the normal diet the blood acetone varied only slightly with work, always within the normal range (0.51 to 1.25 mgm. per cent).

On the ketogenic diet initially high blood acetone levels (2 to 5 mgm. per cent, and on one occasion 14.5 mgm. per cent) showed a considerable drop (3.01 ± 0.89 mgm. per cent for 17 trials[3]) during moderately heavy work (0.097 H.P. for 15 min.) with a considerable rise two hours later (6.15 ± 1.08 mgm. per cent). During less heavy work (0.08 H.P. for 30 min.) the drop was less, but still significant (0.72 ± 0.099 mgm. per cent) and it was followed by a non-significant rise (6.84 ± 4.46). During more intense work (0.495 H.P. for 43 sec. and 0.182 H.P. for 5 min.) there was no drop.

Blood glucose during exercise showed a significant drop on a normal diet (14.33 ± 4.18 mgm. per cent for 6 estimations), but no significant drop on the ketogenic diet (1.5 ± 2.46 mgm. per cent for 12 estimations). The difference between these changes (12.83 ± 4.87 mgm. per cent) indicates a significant change.

Lactate after exercise was increased in proportion to the severity of work or in inverse proportion to the time after onset of work (73.5 mgm. after 5 min. at 0.182 H.P., 48 mgm. after 15 min. at 0.097 H.P., and 25.5 mgm. after 30 min. at 0.08 H.P.). The changes in lactate and pyruvate were similar on both diets. These are shown in figure 1, which also illustrates the typical changes in acetone and glucose in both subjects working at 0.097 H.P. for 15 min. for three trials at 2 hr. intervals.

The hemoglobin levels serve to illustrate that the changes could not be attributed to hemoconcentration or dilution. The high glucose level in D. R. about 4 hours after the last exercise on the normal diet is attributable to a meal 1 hour before this estimation. The comparable meal in A. N. was before his third trial when he showed his second highest glucose level. A. N.'s highest glucose level was 1 hour after his evening meal. Meals while on the ketogenic diet were taken at comparable times to those on the regular diet but contained very little carbo-

[3] These changes are expressed as mean and standard error. The test of significance is a ratio of mean change to its standard error (Fisher's t) such that P is less than 0.05 for significant and less than 0.01 for highly significant (18).

hydrate. Estimations made while working have been omitted from the figure in the interests of simplicity. They were generally intermediate between the before and after readings except for lactate which was sometimes higher at 5 min. than at 15 min.

Guinea-pig experiments. Some of these results are illustrated in table 1. It is of interest that the fatigue times were just as long for fasted guinea pigs as for fed ones.

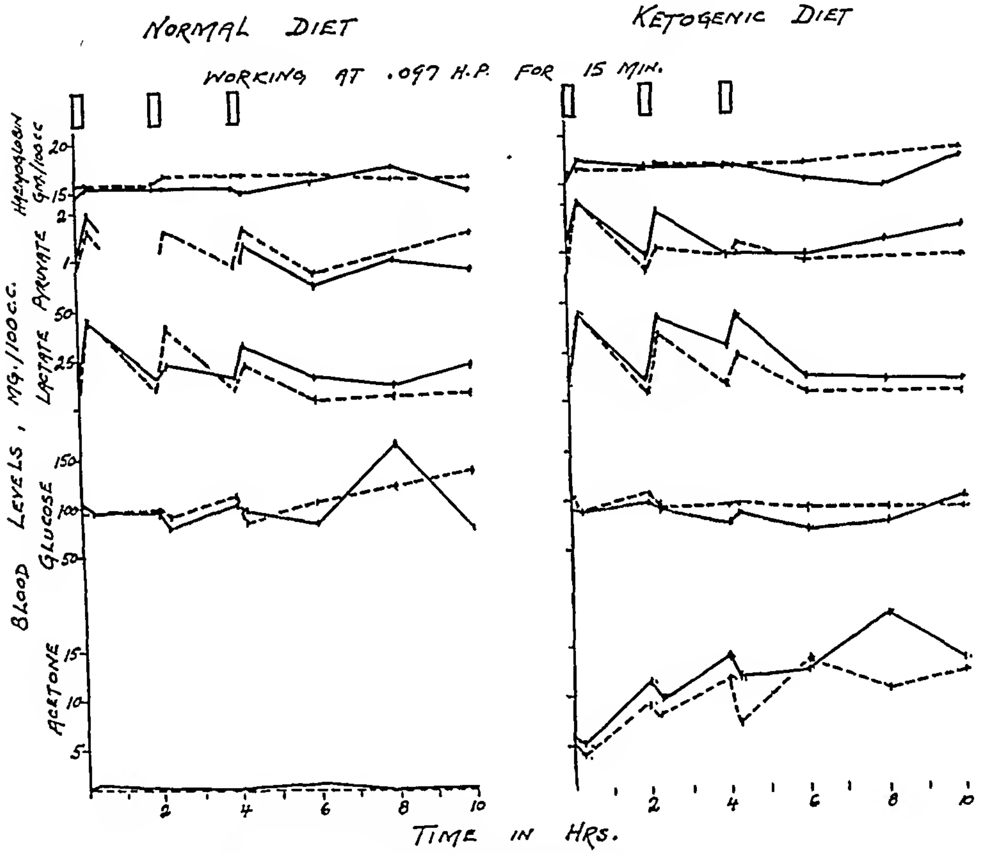

Fig. 1. Blood changes in two human subjects while working on a bicycle ergometer and during the subsequent recovery period (subject D. R., continuous line; subject A. N., broken line).

The significant differences in the fasted animals from the fed ones are as follows: initial blood acetones higher (t = 3.65) and muscle and liver glycogen lower (t = 4.17 and 13.0); rise in blood acetones at fatigue greater (t = 3.83); rise in blood lactate at 10 min. less (t = 3.11); drop in muscle and liver glycogen less (t = 3.47 and 9.70). The slightly lower blood acetone at 10 min., the slightly higher blood glucose at fatigue and the slightly less drop in blood glucose in the fasted animals are not significant, although they tend in the direction of the findings in the human experiments.

Results from animals sacrificed at other times previous to fatigue and after various periods of recovery have been omitted from the table to avoid complexity. These results indicated that the peak of lactate and pyruvate level was at around 10 min. of working and that this was followed by a lower plateau previous to a second rise at fatigue. There was an appreciable rise of ketone bodies during recovery.

TABLE 1

Blood and tissue changes in guinea pigs while running on a motor driven treadmill

	FED			FASTED		
	Control	10 mins.	Fatigue	Control	10 mins.	Fatigue
	No. of animals					
	7	7	5	4	3	3
	Time to fatigue (min.)					
	184 ± 24			219 ± 25		
Blood						
Acetone (mgm./100 cc.).....	0.99 ± 0.114	1.41 ± 0.163	9.07 ± 1.24	2.77 ± 0.475	1.76 ± 0.38	20.90 ± 2.82
Sugar (mgm./100 cc.).....	126.7 ± 6.10	128.1 ± 4.85	60.6 ± 2.05	111.5 ± 6.39	128.6 ± 4.63	7.20 ± 8.08
Lactic (mgm./100 cc.).....	18.1 ± 1.66	64.8 ± 9.93	46.7 ± 9.65	13.5 ± 1.40	33.65 ± 1.25	26.2 ± 2.5
Muscle						
Lactic (mgm.%)....	23.7 ± 2.08	117.4 ± 11.72	61.5 ± 12.24	22.86 ± 3.64	76.18 ± 18.62	38.7 ± 9.44
Glycogen (mgm.%)....	923 ± 70.4	596 ± 59.0	168 ± 19.0	584 ± 17.3	438 ± 32.2	102 ± 24.8
Liver glycogen (%).........	5.79 ± 0.42	5.67 ± 0.52	0.38 ± 0.17	0.17 ± 0.10	0.14 ± 0.08	0.033 ± 0.009

Discussion. The performance of moderate work by the two human subjects studied while on a normal diet was associated with a significant drop in blood glucose level. One may assume that this was the result of an increased flow of glucose into the muscles to replenish the glycogen stores. Continued moderate work would thus entail a decreasing liver glycogen level as a result of the change: liver glycogen → muscle glycogen → lactic acid. This appears to be substantiated in the observations that the fed guinea pigs, following exhaustive muscular exercise, had lost almost their entire supply of liver glycogen. This strongly suggests that in the presence of sufficient carbohydrate stores the oxidation of lactic acid during muscle contraction proceeds at a rapid rate. As a matter of fact, Dill and his associates (19) were able to demonstrate a dependence of the rate of lactic acid removal on the rate of metabolism. The changes in blood pyruvate, a substance known to be an intermediate in the breakdown of carbohydrate still further supports this view.

The performance of moderate work by the human subjects while on a ketogenic diet was not associated with a drop of blood glucose. It was associated, however,

with a significant disappearance of blood ketone bodies. Following cessation of work blood ketone bodies increased, presumably as a result of the increased production of these substances by the liver, associated with the sudden stop of utilization. The results obtained with the fasted guinea pigs are essentially the same. The small discrepancies between the findings in the humans and the guinea pigs can be accounted for by the fact that the humans, although in ketosis, were not fasted and may have had larger glycogen stores as compared to their own controls than did the fasted guinea pigs.

In the human experiments the more strenuous exercise for 45 sec. and for 5 min., which produced the greatest increase in lactate, did not result in any decrease in blood ketone bodies. A portion of the energy expanded was probably supplied anaerobically and the muscles were dependent on the "lactic acid mechanism" for contracting in an oxygen debt. Presumably this, together with the available lactic acid, was sufficient to meet the energy requirements.

SUMMARY

A study has been made on human subjects while on normal and on ketogenic diets, on the effect of work (bicycle ergometer) on the blood ketone bodies, glucose, lactate, pyruvate and hemoglobin. A similar study has been made on fed as well as fasted guinea pigs running on a motor driven treadmill. In these, muscle lactate and muscle and liver glycogen changes also were followed.

Pedalling at 0.097 H.P. for 15 min. while on a ketogenic diet produced a highly significant drop in blood ketone bodies (3.01 ± 0.89 mgm. per cent). At the same time the drop in blood glucose which was shown on a normal diet failed to occur. Cessation of work resulted in a significant increase in ketone bodies. Results were obtained with the fasted guinea pigs, which illustrated a lower utilization of carbohydrate stores for the same amount of work than in the fed ones. It appears from the results reported that ketone bodies can be utilized by muscles and that during exercise their rate of production by the liver is increased. This, however, becomes apparent only during deficiency of carbohydrate stores (fasting, or a ketogenic debt).

We wish to thank Prof. J. B. Collip under whose direction this work was carried out.

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THE EFFECT OF HEMORRHAGE ON NORMAL AND HYPOCOAGULABLE BLOOD AND LYMPH

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The effect of acute hemorrhage on the animal whose blood and lymph are hypocoagulable has not been previously tested, nor has the relationship to the blood of either normal or hypocoagulable lymph been adequately determined. However an increased acceleration in the coagulation time of the blood during rapid progressive hemorrhage has been noted by many observers (1) (2). Although the cause for this change has not been fully established, Drinker et al. have suggested that the lymph might be the responsible contributory factor in this altered coagulability of the blood (3).

In the present investigation, the effect of acute hemorrhage was studied in dogs whose blood was rendered hypocoagulable by a variety of anticoagulants. The plan was to determine quantitatively changes in the clotting time and in the coagulation factors in samples of blood and lymph taken at successive intervals during the course of hemorrhage.

Twenty normal dogs were divided into four groups and were prepared in the manner described below.

Group I. Five dogs were subjected to hemorrhage and lymph drainage.

Group II. Five dogs were given an intravenous infusion of 50 cc. of 1 per cent protamine solution (salmine sulfate) over a period of 15 minutes and then subjected to hemorrhage and lymph drainage.

Group III. Five dogs were injected intravenously with 50 cc. of 20 per cent Witte's peptone solution and were then subjected to successive bleedings and lymph drainage.

Group IV. Five dogs were given an intravenous injection of heparin (10 mgm. per kilo) and subjected to hemorrhage and lymph drainage.

Each dog was anesthetized by receiving an intravenous injection of 1 cc. of 3 per cent pentobarbital sodium per kilo of body weight. The thoracic lymph duct was isolated in the neck, dissociated from its entrance into the subclavian vein, and intubated with a Lindemann needle. Rapid successive bleedings were effected by the withdrawal of 45 cc. of blood from the femoral artery at 20 minute intervals. Lymph was continuously collected and also sampled at 20 minute intervals. One and a half cubic centimeters of $2\frac{1}{2}$ per cent sodium citrate solution was added to each 10 cc. of blood or lymph. In addition for each of the groups described above, two dogs serving as controls were studied under identical conditions but were subjected to the removal of only 5 cc. of blood and lymph at 20 minute intervals during the experimental period. The following deter-

[1] Aided by a grant from the New York Foundation.

minations were made on each sample of blood and lymph; coagulation time (Lee and White method) (4), plasma coagulation time (Howell recalcification method) (5), prothrombin time (Quick method) (6), antithrombin time (7), and fibrinogen concentration (8).

RESULTS. *Group I.* The coagulation time of the blood and lymph usually decreased after each withdrawal of blood. As a result of the repeated bleedings, the clotting time of the blood fell an average of 106 seconds below its initial value, and the clotting time of the lymph was reduced an average of 80 seconds. The coagulation time of the plasma from the blood and lymph followed the same trend as the latter. The prothrombin time and the antithrombin time showed no significant changes in either the blood or lymph. After the first three or four bleedings, the concentration of fibrinogen tended to rise in the blood and lymph but fell markedly as the experiment progressed. In the control dogs for this group, there were no significant changes in the coagulation factors or in the clotting time of either the blood or lymph.

Group II. After the completion of the injection of the protamine solution, the coagulation time of the whole blood and lymph was markedly prolonged, remaining incoagulable for more than four hours. At the end of the experiment, the coagulation time of the blood was reduced to about 10 minutes while the post-injection samples of lymph stayed hypocoagulable. The plasma coagulation time of the blood was increased markedly above its initial value and then fell progressively, while the plasma coagulation time of the lymph remained elevated throughout the entire period of hemorrhage. Prothrombin time and antithrombin time which were prolonged in the blood and lymph after the injection of protamine declined slowly toward their initial values. Also as a result of the injection, there was a marked reduction in the fibrinogen concentration of the blood and lymph which continued at a low level during the course of the experiment. Noteworthy was the rapid and profuse flow of lymph which was induced by the injection of protamine and was not affected by the rapid bleedings which served to slow the flow of lymph in the dogs of group I. In the control dogs, the prolongation of the coagulation time, the prothrombin time, and the antithrombin time of the blood and lymph persisted throughout the entire period of observation with but moderate subsidence of the anticoagulant effect of protamine. The concentration of fibrinogen decreased quantitatively in the blood and lymph of the control animals even though they were not subjected to hemorrhage.

Group III. The intravenous injection of Witte's peptone produced an effect on the clotting time of the blood and lymph and their coagulation factors similar to that produced by protamine. Except for the failure of the coagulation time of the lymph and its plasma to fall below their post-injection values, the clotting time of the blood and the blood plasma fell markedly as a result of the bleedings so that the final values were lower than the initial values and comparable to the effect of hemorrhage in the dogs of group I.

Group IV. After the intravenous injection of heparin, the clotting time, the plasma coagulation time, the prothrombin time and the antithrombin time of

the blood were increased. Heparin acted more directly on the blood and did not appear to affect significantly the flow of lymph or its coagulation factors. Also, the concentration of fibrinogen in the blood and lymph was not markedly affected by the injection of heparin. After repeated bleedings, the final coagulation time of the blood and lymph were reduced below their original values. In the control dogs, the heparin effect on the coagulation factors of the blood and lymph was similar to that described above but subsided significantly as its anticoagulant action wore off. However, the changes were not as marked as in the heparin dogs subjected to hemorrhage.

Discussion. It may be assumed that an increase in concentration of thromboplastin in the plasma was responsible for the hypercoagulability of the blood following hemorrhage, since the responsible factors were apparently neither

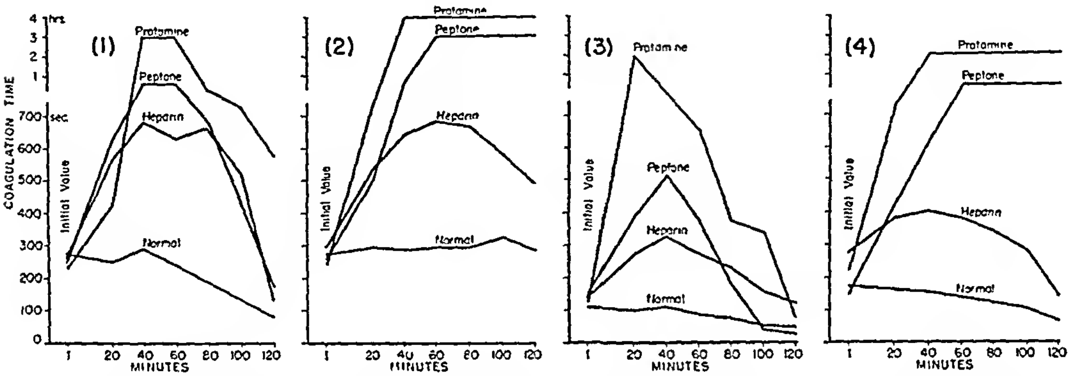

Fig. 1 shows the changes in the coagulation time (ordinate) of normal and hypocoagulable blood (protamine, peptone or heparin) during rapid progressive hemorrhage. Figure 2 shows the changes in the coagulation time of normal and hypocoagulable blood in control dogs not subjected to hemorrhage. In contrast with figure 1, the acceleration of the clotting time as a result of hemorrhage does not occur. Figure 3 shows the changes in the coagulation time of normal and hypocoagulable blood plasma during hemorrhage. Plasma coagulation time and the clotting time of the blood parallel each other. Figure 4 shows the effect of hemorrhage on normal and hypocoagulable lymph during the course of the experiment. Only the clotting times of the lymph of the normal and heparin injected animals follow the same trend as the clotting time of the blood.

prothrombin, antithrombin or fibrinogen. It may be assumed also that the increased mobilization of thromboplastin was due to a more rapid lysis of platelets (9) as one of the compensatory mechanisms of hemorrhage. The resultant reduction or acceleration of the coagulation time was manifested both in the blood and the plasma during the course of rapid progressive hemorrhage.

The mechanism by which the anticoagulants suppress the coagulability of the blood has not been fully explained. Some authors have claimed that each anticoagulant retards the clotting of blood in a specific manner; i.e., protamine by inactivating thromboplastin by forming a cephalin-protamine complex (10), peptone by increasing the antithrombic titre in the blood (11), and heparin by its antiprothrombic or antithrombic activity (12). Another group of observers have indicated that anticoagulants such as used in these experiments cause a

mobilization of heparin in the blood which inhibits the lysis of platelets thus preventing the liberation of thromboplastin and retarding coagulation (11). However, regardless of the method of inhibition of the coagulability of the blood, the response of the clotting mechanism to acute hemorrhage was usually the same as in the uninjected animals of group I, namely, an acceleration of the coagulation time and a trend toward hypercoagulability. The controls in which the animals were not subjected to hemorrhage and in the others whose blood was rendered hypocoagulable failed to show this response.

The results of these experiments support similar clinical observations made by Sahli (13) who observed that hemorrhage in hemophiliacs resulted in a reduction of the prolonged coagulation time of the blood, and by Lawson and Graybeal (14) who found that by the routine therapeutic withdrawal of blood, the frequency of hemorrhages in a hemophiliac was significantly decreased.

Although no evidence was found to indicate a special function or contribution of the thoracic duct lymph to the coagulating mechanism of the blood, other equally interesting observations were made. The clotting time and the values of the coagulation factors of the blood and of the thoracic duct lymph differed quantatitively and did not indicate that any relationship existed between the two on a purely filtration basis. During the course of the hemorrhage experiments, the coagulation time of the lymph in the normal and heparin injected animals followed the same pattern as that of the blood, while in the protamine and peptone series, the lymph remained hypocoagulable in spite of hemorrhage and the reduction of the clotting time of the blood. The latter hypocoagulability may be accounted for on the basis of a dilution effect since the rate of flow and the quantity of lymph were greatly increased. In the present experiments, it was possible to obtain a hypocoagulable blood (heparin group) without markedly affecting the clotting time of the lymph, and Shore (15) showed that it was also possible to render the lymph hypocoagulable without affecting the coagulability of the blood.

SUMMARY

1. Rapid progressive hemorrhage rendered the blood and lymph of normal animals hypercoagulable.

2. Rapid progressive hemorrhage also caused a marked reduction of the clotting time in animals whose blood was experimentally rendered hypocoagulable.

3. The mechanism for increased coagulability of the blood during hemorrhage was related to the increased mobilization of thromboplastin.

4. The lymph of protamine and peptone injected animals remained hypocoagulable in spite of hemorrhage.

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DRUG ACTIONS ON THE SPONTANEOUSLY BEATING TURTLE VENTRICLE INDICATING LACK OF INNERVATION*[1]

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Since the pronouncement of Gaskell (1900), evidence tantamount to proof has accumulated that the turtle ventricle is not innervated by inhibitory vagus fibers. The relations of the sympathetic nerves to this tissue are not equally clear. In this report we offer evidence to indicate that the turtle ventricle is without either sympathetic or parasympathetic nerves.

Garrey and Chastain (1937) have already shown that eserinized ventricular strips from the turtle heart, caused to contract by regular electrical stimuli, show no inhibition when treated with acetylcholine. They suggested that this indicated a lack of cholinergic nerves to the tissue. We have repeated this observation on ventricular strips beating spontaneously. We have also tested such automatically active strips with a number of the so-called autonomic drugs, sympathomimetic as well as parasympathomimetic, without obtaining any evidence of neural control.

METHODS. Thin strips of turtle ventricle, usually from the apical half, were suspended in a cup containing a physiological saline so that their contractions were recorded on a kymograph. Oxygen was bubbled vigorously through the solution during the whole time of observation. As Howell (1901) has reported, these ventricular strips do not, as a rule, beat spontaneously in the animal's own serum or in an equivalent salt solution. He has indicated that this is due to the concentration of potassium present. In our physiological saline for turtles, which contains considerably less potassium than most turtle serum, according to the analyses of the latter made by Smith (1929), the strips usually began contracting soon after they were immersed. This turtle saline consists of 120 mM NaCl + 2.5 mM KCl + 2.5 mM $CaCl_2$ per liter of water alkalinized to a pH of approximately 7.8 with $NaHCO_3$. Some strips contracted regularly from the beginning, but most contracted irregularly. They could be caused to beat regularly by transferring them to a solution containing less potassium or by soaking them in the saline in the refrigerator for several hours. Strips left overnight or for several days, immersed in this saline, usually beat regularly. We have occasionally used strips which had been seven days in the refrigerator.

Drugs were added in alkaline solution to the bath where they were evenly distributed by the stirring action of a stream of oxygen.

Usually the auricles from the same heart were also suspended in the bath to record separately the action of drugs on innervated tissue and thus control drug

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potency as well as other conditions. It should be explained that the strips of ventricle are not only thin but also spongy in structure, so that materials can diffuse into them as easily as they enter the auricular tissue.

Finally the auricles and the ventricle of the frog heart were suspended separately in the same solution and subjected to the action of the same drugs. The ventricle of the frog is known to have both sympathetic and parasympathetic nerve supply to the myocardium. Its reactions to autonomic drugs are, in contrast to those of the turtle ventricle, like those of the turtle auricle. This adds convincingly to the evidence indicating the turtle ventricle to be without autonomic nerves to the myocardium.

RESULTS. *Acetylcholine.* After being treated with eserine (usually 1:1,000,000), the tissues were exposed to acetylcholine in concentrations up to 1:10,000. In concentrations several times greater than those required to completely inhibit the auricular beat, there was no inhibition of the ventricular beat, which was unaffected (fig. 1). The frog ventricle was inhibited by acetylcholine in the same manner as the auricle (fig. 2).

Pilocarpine. In concentrations far in excess of those which cause complete cessation of beat in the auricle, pilocarpine had no effect on the ventricular beat (fig. 1). The frog ventricle again was inhibited in the same manner as the auricle (fig. 2).

Adrenaline. The ventricular strips were treated with adrenaline in various concentrations from 1:25,000,000 to 1:10,000. In concentrations which cause a maximal acceleration of the turtle heart and an increase in the amplitude of its auricular beat, adrenaline caused only a slight increase in the amplitude of the ventricular contraction without any marked increase in rate (fig. 1). This increase in amplitude varied in different preparations from nothing at all to a maximum of 15 per cent, whereas the auricles and the frog ventricle (fig. 2) will often show an increase in amplitude of 100 per cent. The effect on the turtle ventricle decreases with time after removal from the animal. Furthermore, it is difficult to get the adrenaline effect repeatedly on the turtle ventricle, since the increase in amplitude is not so readily reversed in fresh saline as it is in the auricles and in the frog ventricle. All this suggests that the ventricle of the turtle does not react to adrenaline as do the turtle and frog auricles and the frog ventricle because it does not possess sympathetic nerves nor the receptor substance which accompanies innervation. This would mean that the slight increase in amplitude which the ventricular contractions show must be due to a direct effect on the cell, in the sense that it does not require a special reaction at the neuromuscular junction.

Ephedrine. Ephedrine had the same action as adrenaline (figs. 1 and 2).

Potassium excess. The beat of a fresh ventricular strip is stopped by a very small excess of potassium but strips which have been kept for some time in saline in the refrigerator are much more resistant to the action of potassium, becoming more like the auricles and the frog ventricle in this respect. This action of potassium can be quickly reversed on returning to the original solution. The effect is not on the contractile mechanism because the beat stops abruptly without any

weakening of the contraction, and the occasional beats which occur after the rhythm has been stopped are usually of normal amplitude. Furthermore, one of us (W. E. G.) has unpublished records showing that the electrically stimulated ventricle is not thus arrested by low concentrations of potassium of this order but instead behaves like the auricle, showing a gradual decrease in amplitude with increasing potassium chloride concentrations beginning around 100 mgm. per cent. The effect of potassium on the spontaneously contracting strips is there-

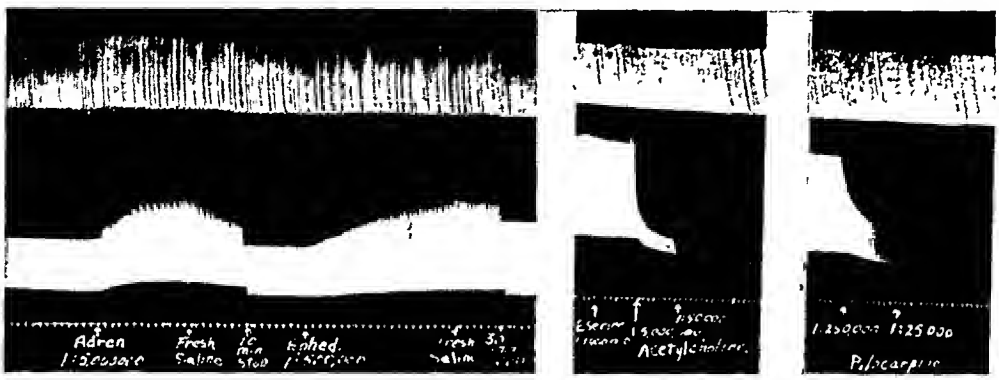

Fig. 1. Kymograph tracings of the contractions of strips of the auricle and the ventricle from the turtle heart showing the effects, from left to right, of adrenaline (1:5,000,000), ephedrine (1:500,000), acetylcholine (1:5,000,000 and 1:50,000) after eserine, and pilocarpine (1:250,000 and 1:25,000). Time = 30 sec. intervals.

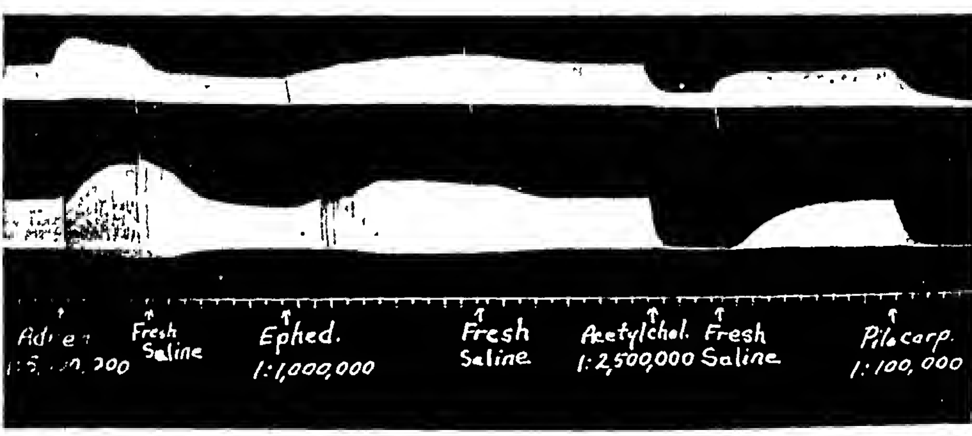

Fig. 2. Kymograph tracings of contractions of the auricles and the ventricle of the frog heart showing, from left to right, the effects of adrenaline (1:5,000,000), ephedrine (1:1,000,000), acetylcholine (1:2,500,000) and pilocarpine (1:100,000). The upper tracing was made by the ventricle, the middle tracing by the auricles and the bottom tracing by the time marker indicating intervals of 30 sec.

fore to stop the pacemaker reaction or to prevent the conduction of the stimulus to the contractile mechanism. Adrenaline and calcium can counteract to some degree these effects of potassium. In this connection it should be pointed out that acetylcholine and potassium, which are similar in so many of their effects, differ markedly in their action on these ventricular strips and hence in the mechanism by which their effects are produced.

Calcium excess. Excesses of calcium cause an increase in the amplitude of the contraction of both auricle and ventricle in the turtle as well as in the frog; in accordance with our hypothesis this represents an effect directly on the cell.

DISCUSSION. Studies on tissues without nerve supply give promise of adding to our knowledge of the site of action of the autonomic drugs, particularly in the gap between the production of chemical mediators at nerve endings and the specific response of the effector. This gap is at present filled only with the term "receptor substance." Unfortunately the reports of work in this field have not always shown agreement. (See Euler (1938) and Armstrong (1935) for literature.) The gist of the matter seems to be that whereas non-innervated tissue (embryonic heart, placental blood vessels, etc.) may react to autonomic drugs, they usually do so only at high concentrations. It seems certain that innervation confers a special sensitivity to these drugs, which may be due to the development of a receptive substance at the neuromuscular junction. This sensitivity, as is well known, persists after the nerves have been sectioned and have degenerated, so it must be intimately connected with the muscle cells. (Cannon and Rosenblueth, 1937.)

Of the agents we tested, adrenaline, ephedrine, potassium and calcium affected the contraction of the turtle ventricle in low concentrations. If our evidence mentioned above be accepted as proof of the lack of innervation in this tissue, then these agents can be considered as having a direct action on the myocardial cells in the absence of any specific receptor substance associated with innervation. Adrenaline and ephedrine, however, have a much greater effect in the presence of innervation, whereas the effect of potassium in stopping the beat was most evident on the non-innervated turtle ventricle.

SUMMARY

1. Isolated strips of the ventricle of the turtle heart will beat spontaneously and regularly if bathed in a well oxygenated physiological saline solution containing potassium and calcium in equivalent molar concentration but having less potassium than turtle serum.

2. These ventricular strips do not show inhibition either of impulse initiation or contractility when treated with acetylcholine (plus eserine) or pilocarpine in concentration far in excess of those required to inhibit the auricle and the frog ventricle. This confirms previous work indicating that the turtle ventricle lacks a vagus nerve supply.

3. Adrenaline and ephedrine cause a small increase in the amplitude of the contractions of ventricular strips but the effect is quite small compared with the effect on the auricle and on the frog ventricle. It is suggested that this indicates a lack of sympathetic nerve supply.

4. A small excess of potassium, which has little effect on the auricle or the frog ventricle, causes cessation of the beat of fresh turtle ventricle strips. The effect is not on the contractile process. The sensitivity of these strips of turtle ventricle to potassium decreases with time after excision.

5. Calcium excess causes an increase in the amplitude of both auricular and ventricular contractions.

6. It is indicated that acetylcholine, pilocarpine, adrenaline and ephedrine have their principal effects on the heart muscle only through the receptor substance which accompanies innervation. Adrenaline and ephedrine have a slight stimulating action on the aneuric myocardial tissue. Potassium and calcium appear to have a definite action directly on the myocardial cells lacking the receptor substance.

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RESPONSES IN SIZE, OUTPUT AND EFFICIENCY OF THE HUMAN HEART TO ACUTE ALTERATION IN THE COMPOSITION OF INSPIRED AIR[1]

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Cardiovascular responses to acute hypoxia have been extensively studied but fundamental data from rigidly controlled experiments on man are still not numerous. It is well known that sudden change to oxygen partial pressures of 90 mm. Hg or less causes acceleration of the pulse which is increasingly marked with decreasing pO_2. A bluish pallor and cold extremities are noted. When persons are maintained in this state for some minutes or hours they may suddenly collapse in syncope with an almost imperceptible pulse or they may develop marked complaints of dyspnea, "pounding heart," and violent headache. Occasional "heart attacks" and even sudden death in "normal" people at high altitude are sometimes mentioned.

It is not surprising, therefore, that it is frequently believed hypoxia imposes a severe strain on the heart and that many of the important symptoms of hypoxia are referable to either cardiac insufficiency or to an adaptive hypertension. In support of this view is the fact that cardiac dilatation is readily produced by anoxia in isolated hearts and in acute animal experiments and that severe exertion plus the chronic hypoxia of high mountains is reported to produce dilatation of the heart in man (Van Liere, 1927; Spycher, 1931).

It should be noted, however, that critical data on man are absent or extremely meager for: 1, the size of the heart; 2, the stroke output and minute output; 3, the relative efficiency of the heart in the hypoxia condition. Studies on these and related questions on 27 normal subjects are reported here.

Procedure. Healthy male subjects between the ages of 18 and 30 years were studied. All were free from signs of cardiovascular abnormality. All experiments were made in a constant environment room (78°F., humidity 45 per cent to 55 per cent relative saturation). The subjects were quietly seated in front of the roentgenkymograph throughout the experiments.

The subjects breathed through an anesthesia mask supplied with, successively, room air, the test gas mixture, and room air. Krogh type low-resistance diaphragm valves were used. The total respiratory dead space of the apparatus system was 160 cc. Supply and collection gasometers were well-balanced and compensated. In most cases where syncope did not intervene the test gas mix-

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ture was inspired for 15 to 20 minutes (average 17 min.), but in some experiments the periods were shorter (10 to 14 min.) or longer (24 to 48 min.). The initial control and final recovery periods were usually 20 to 30 minutes each.

The partial pressures of oxygen used in the test gas mixtures corresponded to altitudes of 18,000 to 28,000 feet. In some cases carbon dioxide at partial pressures of 14 to 30 mm. Hg was added to the inspired air. In another series of experiments pure oxygen was supplied as the test gas mixture.

Measured variables. Heart rate, blood pressure and respiration were measured at very frequent intervals by recording devices and a team of three observers. Roentgenkymograms were taken 1, immediately before throwing the valve from room air to the test gas; 2, during the last 2 minutes of respiration of the test gas, and 3, after 10 to 25 minutes of recovery. Cardiac volumes in diastole and in systole and stroke outputs were measured and calculated according to the methods developed in this Laboratory (Keys et al., 1940).

Electrocardiographic findings, oxygen consumption, respiratory quotient, and so on were measured in many experiments. None of the latter appeared to provide useful new information and accordingly will not be discussed here in any detail.

Derived variables. Minute volume output of the heart was calculated from the stroke output and the average pulse rate during the minute in which the roentgenkymographic exposure was made.

An index of "*relative cardiac work*" was obtained from the product of the minute volume and the mean blood pressure characteristic of the period in which the minute volume was measured. Similarly, an index of "*relative cardiac effort*" was obtained from the product of the heart rate and the diastolic volume of the heart.

An index of "*relative cardiac efficiency*" is supplied by the ratio of "work" to "effort." Since we are concerned in changes rather than absolute values or comparisons of individuals, such indices should be acceptable if the values in the test period are expressed as percentages of the control values on the same individual.

RESULTS. *Simple hypoxia.* A number of the experiments with simple hypoxia were terminated by the sudden collapse of the subjects. Since these require separate consideration we shall discuss first the experiments in which collapse did not occur.

Blood pressure and pulse records from a typical experiment are given in figure 1. In this experiment the diastolic volume after 13 minutes of hypoxia was 98 per cent of the control volume and the minute volume was 130 per cent of the control. The stroke output was almost exactly the same in both periods. In 15 experiments of this kind with pO_2 between 60 and 80 mm. Hg (average 75 mm.) the pulse rate increased in 10 to 20 minutes from 10 to 28 beats per minute (average +19). There was no consistent change in systolic blood pressure (range −12 to +18 mm. Hg), but the diastolic pressure declined 6 mm. on the average (maximum −24 mm.). The pulse pressure was increased in 12 out of the 15 experiments (average +7 mm., maximum +28 mm.). The minute

volumes, diastolic heart volumes and the ventilations are tabulated, together with the calculated "work," "effort" and "efficiency" in table 1.

It appears that cardiac "work" and "effort" are increased by about 25 per cent on the average, that this is achieved with no increase in diastolic heart size and that cardiac "efficiency" is certainly not impaired. It is interesting that, on the average, ventilation and minute volume are increased to very nearly

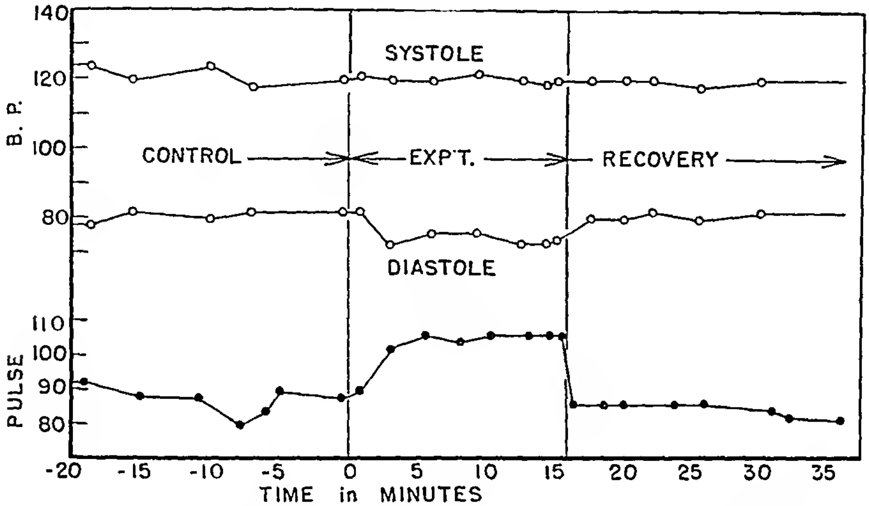

Fig. 1. Blood pressure and pulse rates in a typical low-oxygen experiment (expt. C1–29). During the experimental period the oxygen content of the inspired air was 8.96 per cent. Relative diastolic volume of the heart was 100 at −2 minutes and 98 at +13 minutes. The stroke output of the heart was identical at both of these times.

TABLE 1

Mean changes induced by hypoxia in minute output and diastolic volume of the heart, ventilation of the lungs, and in "work," "effort," and "efficiency" of the heart

All values are for inspiration of air with oxygen at a partial pressure of 64 to 79 mm. Hg and are expressed as percentages of the control levels measured immediately prior to the induction of hypoxia. Barometric pressure averaged 740 mm. 15 experiments.

	PER CENT O_2 INSPIRATION	MINUTE VOLUME	VENTILATION	DIASTOLIC VOLUME	"WORK"	"EFFORT"	"EFFICIENCY"
Mean	10.4	137	132	100	128	123	104
Standard deviation		±31	±24	±6	±20	±8	±15

the same extent—about 35 per cent. In all cases the several variables returned to approximately the initial control levels in 1 to 5 minutes after the resumption of breathing ordinary room air.

Hypoxia with syncope. The recognition of syncope and the timing of its appearance is somewhat difficult in these experiments. Dimness of vision and auditory confusion frequently appeared as did stupor bordering on unconsciousness. In 10 experiments complete anoxic syncope with postural collapse occurred within 2.8 to 15 minutes of the start of breathing the low oxygen

mixture. In these cases there was frequently twitching of isolated muscle groups and slight uncoordinated and apparently involuntary movements of the extremities. These signs were also seen in several instances in which collapse did not occur.

Collapse set in abruptly with few premonitory signs. The blood pressure suddenly fell to zero on the sphygmomanometer and the heart beat became almost imperceptible. Immediately prior to this the pulse was always strong and regular. In several instances sudden hypertension developed just before collapse but this was the exception rather than the rule. In most cases the pulse rate in the hypoxic period before collapse was more rapid than in experi-

TABLE 2

Anoxic syncope

Blood pressures and pulse rates immediately before and in recovery from anoxic syncope induced by respiration of oxygen at partial pressure of 45 to 78 mm. Hg

"Syncope time" represents minutes after start of low oxygen when definite syncope occurred. Values "Before Syncope" were recorded within 30 seconds of collapse. Values in "Recovery" were recorded from 2 to 4 minutes after return to room air.

EXPERIMENT NUMBER	O_2	SYNCOPE TIME	MEAN CONTROL		BEFORE SYNCOPE		RECOVERY	
			Pulse	Blood pressure	Pulse	Blood Pressure	Pulse	Blood pressure
	per cent							
3	9.70	15	92	110/86		/	60	90/70
4	9.70	6.5	76	120/84	106	118/78		
6	10.68	6	78	118/90	94			
21	10.41	6.5	84	108/80	120	108/76	80	114/84
23	10.78	6.5	85	116/80	105	116/66		
40	6.18	2.8	74	122/88	120	145/74	62	125/75
41	6.18	4	82	122/84	140	175/70	64	125/80
42	6.18	3.5	84	122/80	(120)	186/84	(60)	136/80
46	7.75	4	95	120/88	120	112/80	69	120/88
47	7.75	3	80	124/92	118	114/88	60	92/74
Mean...	8.53	5.8	83	118/85	116	134/77	65	114/79

ments where collapse did not occur. Prior to collapse the respiration became shallow and irregular and the appearance was of respiratory failure as the primary cause of collapse.

Naturally it was not possible to obtain roentgenkymographs just before or at the moment of collapse, but any dilatation, if it occurred, had regressed to normal when exposures were made in several cases a few minutes later. It is interesting to note that in all cases there was a relative bradycardia in the early stages of recovery. On recovery none of the subjects seemed any the worse for their experience. There was no memory of events leading to the collapse and shortly thereafter. Data on these experiments are summarized in table 2.

Hypoxia with carbon dioxide. The medical use of carbon dioxide to improve respiration when there is actual or incipient hypoxia is now well established.

Part of the efficacy of respirator systems like the B-L-B mask may be ascribed to the elevation of pCO_2 in the inspired air. The effect of carbon dioxide at a partial pressure of 16 to 24 mm. Hg with oxygen at pO_2 from 56 to 81 mm. was studied in 12 experiments.

The addition of carbon dioxide resulted in marked improvement of the subjects compared with similar or somewhat higher partial pressures of oxygen alone. Syncope only occurred in one experiment. Even in this one case postural collapse was not complete but there were involuntary movements and loss of consciousness after 9 minutes of respiration of 8.55 per cent O_2 plus 2.63 per cent CO_2. In this case the pulse rate rose to 104 from a control level of 84and the blood pressure suddenly rose in one minute from 132/90 to 160/98 and then subsided to 104/70 at the onset of signs of syncope.

In the other subjects there was a moderate increase in heart rate but the

TABLE 3

Mean changes induced by hypoxia plus carbon dioxide in output and diastolic volume of the heart, ventilation of the lungs, and in "work," "effort," and "efficiency" of the heart

All values are expressed as percentages of the control levels measured immediately prior to the induction of hypoxia. Barometric pressure averaged 740 mm.

EXPERIMENT NUMBER	O_2	CO_2	VENTILATION	DIASTOLIC VOLUME	MINUTE VOLUME	"WORK"	"EFFORT"	"EFFICIENCY"
	per cent	*per cent*						
18	9.9	3.34	204	97	110	111	104	107
19	10.0	2.38	178	100	119	130	118	110
22	10.6	2.44	154	94	105	110	104	106
25	11.3	2.22	166	96	103	108	114	95
26	9.5	2.77	192	99	103	111	105	105
28	9.6	2.04	154	99	145	123	111	111
Mean...	10.06	2.63	175	97.5	114	115.5	109.2	105.7

blood pressure changes were small. The subjects remained much more alert and responsive than with simple hypoxia. All of them noticed their increased ventilation which averaged 78 per cent greater than the control level. Several of these same subjects collapsed when breathing oxygen at a slightly higher partial pressure but without any carbon dioxide.

Roentgenkymographic studies were made in 6 of these CO_2 plus hypoxia experiments. The changes of minute volume output of the heart were smaller than in simple hypoxia. There was no change in diastolic heart volume. Both cardiac "work" and "effort" were increased in all cases but to a smaller extent than in simple hypoxia. "Efficiency" was undiminished.

In 6 control experiments CO_2 was administered at partial pressures of 23 to 28 mm. Hg in ordinary air and in oxygen-CO_2 mixtures. The ventilation increased 79 per cent on an average (range +44 per cent to +100 per cent) but the other measured variables were essentially constant except for the pulse rate during respiration of the $O_2 + CO_2$ mixture; in the latter case the pulse rate fell slightly in 3 experiments (range −2 to −9 beats per minute, average −6).

High oxygen. In 7 experiments the test gas mixture consisted of oxygen at a partial pressure of 700 to 715 mm. Hg (corresponding to the case of a diver breathing ordinary air at a depth of around 140 ft.). Pulse rate declined in all cases; there was an average decline, compared to the control period with ordinary air, of 8 beats per minute (range −4 to −14). Also, in all cases there was a very slight rise in diastolic blood pressure, averaging 4 mm. Systolic blood pressure tended to rise very slightly and pulse pressure to fall equally slightly.

There was a small increase in ventilation (average +16 per cent, range +4 per cent to +35 per cent) and a small decrease in minute volume (average −10 per cent, range −20 per cent to +19 per cent). Cardiac "work" appeared to remain constant (average +3 per cent) while "effort" decreased somewhat (average −12 per cent). As a result the apparent "efficiency" of the heart appeared to be improved to a small extent (average +17 per cent, range −9 per cent to +145 per cent).

Discussion. Experiments on operated animals and on surviving hearts show that severe acute hypoxia tends to cause dilatation of the heart. Lorber (1942) has shown that the dilating effect in rabbits' hearts is roughly proportional to the decrease in pO_2 but that this effect only begins below a threshold level of pO_2 which is considerably lower than tolerated by the intact animal. In the experiments reported here there was no tendency to produce any dilatation of the heart in either diastole or systole. It may be suggested that the time periods were too short to allow cardiac dilatation. This is not the case; we have produced well-marked dilatation of the hearts of normal men in equally short periods by means of the drug neo-synephrine (Keys and Violante, 1942).

It is not possible to make rigid generalizations about the behavior of the blood pressure of men subjected to acute hypoxia. Inadequate control of temperature and emotional factors discredits much work on this subject. The present results were obtained under constant temperature and absence of psychic stimuli. We did not find that subjects who faint at one pO_2 show peculiar or abnormal blood pressure responses at a somewhat higher pO_2 which they can tolerate.

Schneider and Truesdell (1921) stated that syncope could be predicted by a decline in systolic and diastolic blood pressure. A similar statement, based on unconvincing data, is made by Schwarz (1936). Besserer (1936) reported that syncope could be predicted by a transient *rise* in systolic blood pressure. The present results show that sycnope itself involves an abrupt fall in blood pressure and that it *may* be preceded by an abrupt rise. Syncope was frequently unheralded by any notable changes in blood pressure. Cerebral asphyxia or anemia can cause syncope and loss of the wink reflex in man within as little as 5 seconds (Kabat, Rossen and Anderson, 1942). In a state of partial anoxia it is understandable then that syncope might occur without warning. A slight shift of blood distribution could be responsible.

Gellhorn has emphasized the importance of the autonomic nervous system in asphyxia and anoxia (1941). In most, but not all, of our subjects extreme peripheral vaso-constriction occurred at the time of collapse and in one case

this was succeeded in the first minute or so of recovery by a peripheral flush fully as intense as we have ever seen produced by atropine. Hypoxia disturbs the stability of the balance between the sympathetic and parasympathetic nervous systems. The initial tendency for the sympathetic system to dominate gives way to parasympathetic dominance which is well marked and occasionally extreme in recovery. Sweating at the time of collapse is common and sometimes very marked. The skin is always moist in recovery. We have mentioned the characteristic bradycardia in recovery after collapse.

Estimation of cardiac output by the foreign-gas method requires changing pO_2 during the course of the measurement. In the hypoxic state small changes in pO_2 produce large changes in oxygenation. It is not certain that this source of error can be quantitatively eliminated; among other things the rate of equilibration with lung and cardiac tissue is unknown. Further the effective pCO_2 at the moment of oxygenation is uncertain and hence no precise calculation can be made for the relation between pO_2 and oxygen saturation. With the foreign-gas method Christensen and Forbes (1937) reported a moderate increase in minute output in hypoxia and Asmussen and Chiodi (1941) reported an average increase of 98 per cent in minute output of 3 subjects.

Ranke (1936), using the pulse wave method, reported increases in minute output in 5 subjects (8 expts.) ranging from 17 per cent to 101 per cent (average 59 per cent). A much more complete study on 10 subjects with similar methods was reported by Herbst and Manigold (1936). At altitudes of 5,000 to 8,000 meters (in a low pressure chamber) the average change in 30 measurements was +11.3 per cent in stroke volume and +41.4 per cent in minute volume. These results are quite consistent with those reported here.

Matthes and Malikiosis (1937) made estimates using a complicated method involving estimations of arterial saturation with an optical system applied to the ear and estimations of saturation of the mixed venous blood by an indirect method. The results of 3 experiments on one person indicated that minute volume is increased at altitudes of 6,000 to 7,000 meters.

All known methods for estimating minute volume in man are subject to some criticism and the method used here is no exception. However, the present method is particularly satisfactory for measurements of *changes* in stroke and minute output and it is not subject to special or systematic errors at low pO_2.

All observations reported here lead to the conclusion that the normal heart is remarkably resistant to hypoxia and that the capacity of the heart to resist this condition is not ordinarily the limiting factor for the intact normal human body. Perhaps of greatest importance is the fact that the heart may be safeguarded in hypoxia by a very marked increase in coronary flow (Wiggers, 1941).

The absence of any anginal or other cardiac complaints was notable in the many experiments in this laboratory. Persons with coronary disease, however, exhibit signs of cardiac embarrassment and suffer precordial pain under similar experimental conditions. The presence of small amounts of carbon dioxide in the inspired oxygen-deficient air has a markedly beneficial effect in most such persons (Barach and Steiner, 1941). This is in agreement with

the effects reported here. Part of the value of carbon dioxide may be in shunting away some circulation from the non-essential periphery (Gellhorn and Steck, 1938).

SUMMARY

1. The results are reported from numerous experiments in which acute hypoxia was produced in 27 normal young men. All studies were made under constant environmental conditions with the exception of the partial pressures of the gases in the inspired air. In most cases the pO_2 corresponded to that at 18,000 to 28,000 feet altitude; in some cases CO_2 at 14 to 30 mm. Hg was present. Some experiments were made with inspiration of pure oxygen. Exposure lasted from 10 to 48 minutes.

2. From roentgenkymographic measurements it is concluded that cardiac dilatation does not take place under these conditions. The stroke volume remains nearly constant in this acute hypoxia and the minute output of the heart is increased only slightly more than in proportion to the pulse rate change. Cardiac efficiency is unimpaired.

3. Carbon dioxide increases the altitude tolerance and in hypoxia with CO_2 added the pulse rate increases less than without it. Again, the heart doesnot dilate and the stroke volume tends to remain constant.

4. Respiration of oxygen at 4 to 5 times the normal pO_2 results in a slight decrease in cardiac "work" and "effort" with no significant change in heart size or efficiency.

5. Blood pressure responses have no certain predictive value as to whether syncope is to occur. In the present series complete syncope occurred in 10 cases. There is always a relative bradycardia in recovery from hypoxic syncope.

6. Indications were seen that acute hypoxia disturbs the stability of the autonomic nervous system.

7. It is concluded that, in normal young men, attempts to strengthen or safeguard the heart under hypoxic conditions would serve no useful purpose. The heart does not seem to be the limiting factor in tolerance to acute hypoxia.

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SEGMENTAL MOTOR INNERVATION OF THE TIBIALIS ANTERIOR AND GASTROCNEMIUS-PLANTARIS MUSCLES IN THE DOG

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As a preliminary to a study of the influence of stretch on the changes in skeletal muscle during degeneration and regeneration of the motor nerve supply, determinations were made of the segmental innervation of a set of antagonistic muscles of the hind limb. It was necessary to know the precise innervation of the muscles in order to establish a complete motor paralysis, and at the same time cause a minimal disturbance of the functional integrity of the animal. Accurate knowledge of the motor nerve supply was desired also for use in another phase of our problem, namely, that of producing varying degrees of partial paralysis of the muscles selected for study. The purpose of this paper is to report the segmental innervation of the anterior tibial and the gastrocnemius-plantaris complex in the dog.

Method. The right anterior nerve roots of L 3, L 4, L 5, L 6, L 7, S 1, S 2 and S 3 were isolated and a silk ligature tied about each motor root close to its exit from the dural canal. After sectioning the attachment of the roots to the spinal cord, a segment of 1 to 2 cm. in length was removed from the corresponding posterior nerve root. As a rule, the spinal nerve roots of the opposite side were transected. The tendon of the anterior tibial muscle was ligated and freed from its place of insertion, and those of the gastrocnemius-plantaris muscles were tied with a common ligature and detached from the tuberosity of the calcaneus. The tendinous extension of the biceps femoris which accompanies the gastrocnemius-plantaris tendons was isolated and cut. The tendons of these antagonistic muscles were attached to two calibrated isometric levers and adjusted to record simultaneously the tension myograms. Records were made of the muscular responses resulting from the stimulation of each anterior nerve root extending from L 3 to S 3 inclusive. The stimulating current was supplied by a Harvard inductorium.

Results. Observations were made on a group of sixteen dogs. Satisfactory results were obtained in fourteen of the sixteen animals. Table 1 shows the qualitative, semi-quantitative and quantitative data obtained in these experiments. Examination of the data shows that the motor nerve supply of the gastrocnemius-plantaris muscles was derived from the roots of L 5, L 6, L 7 and S 1. Root L 5 was involved in only four of the sixteen animals, and only in dog 1 was it markedly involved This dog had no sacral representation. The motor nerve supply of the anterior tibial muscle was carried by L 6 and L 7 in all animals except dog 1. In this animal the nerve supply of the anterior tibial was confined to L 5. It is interesting to reiterate that this animal showed the

antagonistic muscles to be supplied by L 5, L 6 and L 7 with no sacral representation. Apparently dog 1 was an example of what Sherrington (1892) has termed a "prefixed" animal. Quantitative data obtained from measurement of the myograms are shown for the last six animals listed in table 1. The figures signify the amount of tension developed during muscular contraction and expressed in grams.

TABLE 1

Qualitative, semi-quantitative and quantitative data showing the magnitude of muscular contractions resulting from stimulation of the anterior nerve roots of lumbo-sacral nerves, L 3, L 4, L 5, L 6, L 7, S 1, S 2, and S 3

0 = not tried; − = negative response; +, ++, +++, ++++ = semi-quantitative results referring to the relative magnitude of muscular contractions; figures 411, 3526, etc. = quantitative data expressed in grams tension as revealed by the tension myograms.

DOG NO.	GASTROCNEMIUS-PLANTARIS MUSCLES								ANTERIOR TIBIAL MUSCLE							
	Lumbar segment					Sacral segment			Lumbar segment					Sacral segment		
	Root no.					Root no			Root no.					Root no.		
	3	4	5	6	7	1	2	3	3	4	5	6	7	1	2	3
1	0	−	++	++++	+++	−	−	0	0	−	++++	−	−	−	0	0
4	0	−	0	++++	++++	0	0	0	0	−	0	++++	++	0	0	0
6	0	−	+	++	++++	+++	−	−	0	−	−	++++	++	−	−	−
7	0	0	0	++	++++	++	0	0	0	0	0	++++	++	−	0	0
8	0	−	−	++	++++	++++	0	0	0	−	0	++++	+	−	0	0
10	−	−	−	+	++++	++	−	0	−	−	−	++++	+	−	−	0
11	0	−	−	+	++++	++++	−	−	0	−	−	++++	+++	−	−	−
12	0	−	−	−	+++	++++	−	−	0	−	−	++	++++	−	−	−
21	0	0	−	+	++++	++++	−	−	0	0	−	++++	+	−	−	−
	0	0	−	926	3466	3402	−	−	0	0	−	3174	784	−	−	−
22	0	0	−	++	++++	+++	−	−	0	0	−	++++	++	−	−	−
	0	0	−	1529	4801	4155	−	−	0	0	−	2978	1254	−	−	−
23	0	0	−	+	++++	+++	−	−	0	0	−	++++	+	−	−	−
	0	0	−	818	4091	2627	−	−	0	0	−	2625	411	−	−	−
24	0	0	−	+	++++	+++	−	−	0	0	−	++++	+	−	−	−
	0	0	−	538	3445	2218	−	−	0	0	−	3526	882	−	−	−
25	0	0	+	++	++++	++	−	−	0	0	−	++++	+	−	−	−
	0	0	431	1055	3230	1077	−	−	0	0	−	3095	392	−	−	−
67	0	−	+	+−	+++	++++	−	0	0	−	−	++++	++++	−	−	0
	0	−	650	875	5150	6888	−	0	0	−	−	3526	3690	−	−	0

In this group of animals the total tension developed by the anterior tibial in each instance resulted from stimulation of L6 and L7, the tension ranging from 392 to 3690 grams. The percentage tension developed by the stimulation of L 6 ranged from 48.6 per cent to 86.4 per cent, while that developed by the stimulation of L 7 ranged from 13.6 per cent to 51.4 per cent. The tension developed in the gastrocnemius-plantaris as a result of stimulating the anterior nerve roots showed a wider range of distribution. In the majority of instances the total tension was developed by the stimulation of three anterior nerve roots,

namely, L 6, L 7, and S 1, with the exception of two of the six animals in which it was necessary to stimulate four anterior nerve roots, namely, L 5, L 6, L 7 and S 1. In all animals the majority of the tension was obtained from the stimulation of L 7 and S 1, while lesser amounts were developed from the stimulation of L 6 and L 5. The tensions developed from the stimulation of L 5 ranged from 0 to 650 grams, for L 6—818 to 1529 grams, for L 7—2218 to 5150 grams, and for S 1—1077 to 6888 grams. The percentage of the total tension developed from the individual stimulation of the respective nerve roots in this group of six dogs may be summarized as follows: L 5 = 0—0—0—0—7.4 per cent—4.8 per cent; L 6 = 11.9 per cent—14.6 per cent—10.9 per cent—8.7 per cent—18.2 per cent—6.3 per cent; L 7 = 14.5 per cent—45.8 per cent—54.3 per cent—55.5 per cent—55.8 per cent—38.1 per cent; S 1 = 43.6 per cent—39.6 per cent—34.8 per cent—35.8 per cent—18.6 per cent—50.8 per cent. It is obvious that there was a variable percentage of tension produced in the gastrocnemius-plantaris muscles as a result of stimulation of the lower lumbar and upper sacral nerve roots. The individual variation and the amount of tension developed in different animals was found to be so great that it is impossible to draw any definite conclusions regarding the per cent of total tension supplied by each individual anterior root. However, it is reasonably safe to say that from 14.5 per cent to 55.5 per cent was supplied by L 7, from 18 per cent to 51 per cent by S 1, less than 20 per cent by L 6 and less than 10 per cent by L 5. The mode of percentage tension for L 7 ranged between 40 per cent and 50 per cent, and the mode for S 1 ranged between 30 per cent and 40 per cent.

CONCLUSIONS

From an investigation of the segmental motor innervation of the anterior tibial and gastrocnemius-plantaris muscles of fourteen dogs, the following facts were found:

1. The tibialis anterior muscle in the dog received its segmental motor nerve supply from L 5, L 6 or L 7. In the majority of animals two roots were concerned, L 6 and L 7, with most of the fibers from L 6. Three exceptions were noted; in one, root L 5 alone was involved, in another, most of the fibers were in L 7, while in the third, they were almost equally distributed between L6 and L 7.
2. The segmental motor nerve supply of the gastrocnemius-plantaris complex arose from roots L 5, L 6, L 7 and S 1. In nine animals three adjacent roots were involved. Eight of these received fibers from L 6, L 7 and S 1 while the other one involved L 5, L 6 and L 7. In three animals all four roots were involved (L 5, L 6, L 7 and S 1), and in two animals only two neighboring roots were concerned—L 6 and L 7 in one, and in the other L 7 and S 1.
3. As a rule most of the motor fibers of the gastrocnemius-plantaris complex were carried in two adjacent roots, namely, L 7 and S 1 This was so in ten animals. In two animals the majority of the fibers were supplied by L 6 and L 7, while in the remaining two dogs, the major portion of the fibers were carried in L 7, and the remainder of the fibers equally distributed in L 6 and S 1.
4. The experiments show that it is necessary to transect the anterior nerve

roots of L 5, L 6, L 7 and S 1 in order to insure the development of a complete paralysis of the anterior tibial and gastrocnemius-plantaris muscles of the dog.

5. As regards the ankle flexors and extensors of the hind leg, it is impossible to express a reliable percentage of total innervation that is supplied by each individual anterior nerve root.

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HUMORAL INTERMEDIATION OF NERVE CELL ACTIVATION IN THE CENTRAL NERVOUS SYSTEM[1, 2, 3]

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The comprehensive rôle which the acid humoral mechanism of nerve cell stimulation (Gesell, Brassfield and Hamilton, 1942) may play in the integrations of the nervous system as a whole rests on the soundness of the theory of humoral intermediation of nerve cell stimulation in the centers, a view not generally accepted. Best and Taylor (1939) believe that "Little *direct evidence* (Italics ours) can be cited in support of the concept though certain suggestive observations have been cited," and Bard (1941) believes "While there is almost universal agreement that immediate control of the slowly acting effectors by autonomic fibers is mediated by chemically specific substances liberated at the nerve terminations the evidence bearing on central synaptic transmission certainly favors the electrical theory." The reluctance of physiologists to accept central humoral intermediation on the basis of present knowledge (frequently cited references in textbooks, reviews and monographs) indicates the need of further experimentation if that concept is to play a rôle in the physiology of the central nervous system.

PROCEDURE. Dale and his associates (1929) established the humoral mechanism in simple cholinergic systems by a systematic application of elementary physiological principles: 1, the reproduction of physiological activity by acetylcholine; 2, the potentiation of indirect stimulation by an anticholinesterase; 3, the liberation of acetylcholine by indirect stimulation. We have followed the procedure of Dale and his group and added modified approaches suited to the peculiar intricacy of central nervous activity. The present discussion includes procedures 1 and 2 only.

Our observations were limited almost exclusively to the respiratory act of the dog. They were made under chloralose anesthesia, morphine urethane anesthesia and decerebration performed under evipal anesthesia.

The choice of the respiratory act possessed outstanding advantages—the abundant information on the location, structure and activity of the respiratory center, the automaticity, the even rhythm and intensity of discharge, and the ease of recording respiratory activity. Though one of the simplest of motor integrations the respiratory act possesses the main elements of completeness. Two antagonistic half-centers alternately activate and inhibit the inspiratory and expiratory muscles due to an effective mechanism of reciprocal innervation.

[1] Preliminary report: J. Worzniak and R. Gesell. This Journal Proc. 123: 222, 1938.

[2] Preliminary report: E. T. Hansen, J. J. Worzniak and R. Gesell. Fed. Proc. 1: 36, 1942.

[3] Supported in part by a grant from the Rockefeller Foundation.

These half-centers react delicately to chemical stimuli and to well-known sensory drives. Some of these drives run in specialized nerves and thus permit analysis of specific reflex activities in relation to the neurohumoral theory. Finally, the precise information in the activity patterns of normal breathing offers a criterion of comparison which can leave no question of whether or not duplication of normal central nervous integration has been attained by artificial administration of extrinsic acetylcholine.[4]

Results. 1. *The reproduction of physiological activity by acetylcholine.* An increase in the intensity of breathing is demonstrated with several methods of administration of acetylcholine, intravenous, intra-arterial (cerebral arteries) and intraventricular onto the floor of the fourth ventricle. Injected intravenously, as in figure 1A, an augmentation of breathing occurs which in a few minutes subsides to normal. This response might well be a combined effect of acetylcholine upon the chemoceptors and the respiratory center, for Heymans et al. (1936) have demonstrated that the carotid body is highly sensitive to acetylcholine. Such a double site of action of acetylcholine is illustrated in figures 1B and 1C. In figure 1C, 0.1 mgm. of acetylcholine was injected into the right common carotid artery after denervation of the corresponding carotid body to limit its effects to the center only. In figure 1B the same amount of acetylcholine was injected into the left carotid artery with the innervation of the corresponding carotid body intact. This larger response is, therefore, regarded as the sum of peripheral and central excitation. v. Euler, Liljestrand and Zotterman (1941) "look upon the sinus region as a sort of nervous center with a peripheral localization of the same general type as in the olfactory or optical peripheral organ." Nerve cell activation is thus involved in peripheral as well as central stimulation. Our present paper however deals specifically with the controversial problem of central intermediation.

To establish a purely central action acetylcholine is injected into one of the cerebral arteries, either after denervation of all known respiratory chemoceptors or during cold block of the chemoceptor afferents (Hering's nerves and cervical vagus nerves). This procedure produces a remarkably smoothly co-ordinated hyperpnea (fig. 1D). The precaution required to obtain such results is to avoid excessive doses. If the injection is too rapid, there may be a momentary reduction of breathing similar to that in figure 1C. More often there is a perfect combination of adjustments of increased frequency of breathing, and of increased intensity of both inspiratory and expiratory components, so similar to physiological hyperpneas as to warrant the belief that a truly physiological activity has been produced by an artificial administration of acetylcholine. The shortness of the latent period following injection (about 2 sec.) points unquestionably to a central action but the ultimate proof that the response is a truly physiological activity rests on the basic similarity of the activity patterns of the opposing muscles to those of normal breathing. This point will be established below.

[4] We wish to thank Merck and Company, Inc., for the acetylcholine used in these researches.

2. *On the impossibility of segregating the two dominating central excitatory effects of acetylcholine.* From the observations of others on simple cholinergic systems,

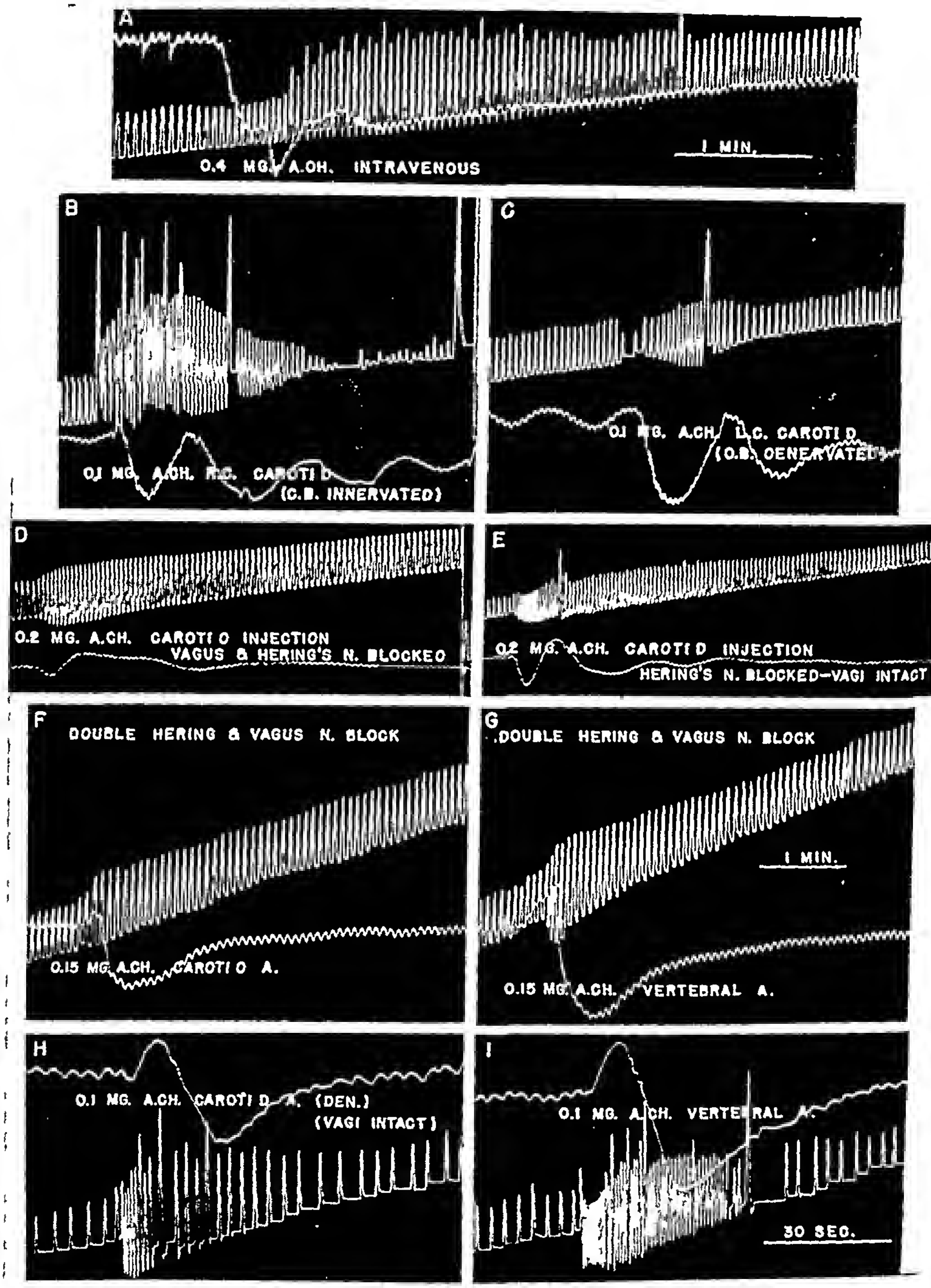


Fig. 1

acetylcholine should exercise two central excitatory effects: 1, a direct excitation at the synapses of the respiratory nerve cell; 2, a potentiation of the effects of all impulses impinging at these nerve cells. If that assumption holds, acetyl-

choline would be expected to produce a varied response of the centers consistent with the general volume and pattern of impinging signals existing at the moment. A comparison of figures 1D and 1E seems to support this view for the experimental conditions differ presumably only in the flow of sensory signals.[5] In 1D all vagal afferent impulses are eliminated by double vagal block whereas in 1E they are returned by vagal deblocking before acetylcholine is injected into the carotid artery. In the latter the frequency of breathing is increased much more and the amplitude is increased much less. Since acetylcholine could hardly have reached the aortic bodies in time or in concentrations to account for the altered response, central potentiation of the highly excitatory vagal proprioceptive reflexes seems to be the likely explanation of the difference of response. This explanation suggests the impossibility of segregating the direct excitatory action of extrinsic acetylcholine from the potentiating action on "intrinsic acetylcholine" (i.e., acetylcholine deposited by nerve impulses). We believe that these two effects actually do work hand in hand in the central nervous systems, under all conditions, experimental or otherwise. The proof of this joint action, through the so-called potentiation of impinging nerve impulses will also be presented below.

3. *The factor of mass stimulation.* Figures 1F and 1G show quantitative differences in the central effects of equal amounts of acetylcholine when injected into the carotid and vertebral arteries respectively. Double Hering nerve and double vagal block eliminate the effects of peripheral chemoceptor stimulation. The larger response (fig. 1G) occurred when acetylcholine was injected into the vertebral artery indicating that acetylcholine injected into the vertebral artery either reaches more nerve cells or reaches them in greater concentration.

If the same comparison of carotid and vertebral injections is made again after re-establishing the pulmonary proprioceptive reflexes by deblocking the vagus nerves (figs. 1H and I), vertebral injections show once more a similar if not greater superiority of stimulation than carotid injections. The much greater hyperpnea produced by vertebral injection could, therefore, be interpreted as the sum of a greater direct stimulation of the center plus a greater potentiation of a greater volume of vagal excitatory proprioceptive signals.

4. *Fundamental implications raised by the central effects of extrinsic acetylcholine.* How can a co-ordinated response to a shapeless stimulus such as arterial injection of acetylcholine be explained? Surely this stimulation must be devoid of such orderly or temporal arrangements emphasized by the electrical theory of transmission. Admitting that sensory patterns and temporal arrangements are wanting, that acetylcholine injected into the vertebral artery reaches the respiratory center and stimulates each and every synapse, and that all of these myriads of stimulations are occurring in a perfectly disorderly and asynchronous manner, what substitute mechanism of nervous integration is there to offer? Activation of heterogeneous synapses would impose a simultaneous excitatory influence upon both half-centers, yet only one-half center discharges at one

[5] We have been unable to relate changes in breathing to blood pressure fluctuations associated with injections of acetylcholine.

time. Simple reciprocating interconnections might provide the mechanism needed to direct this drive. Just as the steam valve in the locomotive shunts the steam pressure (sum total of asynchronous impacts) from one piston to another so is reciprocal inhibition conceived to be the nervous gadget which shifts the relatively steady head of nervous drives (sum total of asynchronously impinging impulses) from one half-center to another and thus converts a chaotic stimulation into an orderly event (Gesell, 1940).

We believe experimental support for this concept of nervous integration is found in the effects of sensory nerve stimulations on breathing, in which either the inspiratory or the expiratory components of dual excitatory afferents (of the vagus and the saphenous and of the chemoceptors) add one to another as one readily adds the stimulating effects of one injection of acetylcholine to another (Gesell and Hamilton, 1941). During the expiratory phase of breathing when the expiratory half-center dominates the respiratory act, one expiratory drive adds to the other, regardless of its origin or of the temporal relation of one group of signals to the other (faradic stimulation from individual coils was used). During the inspiratory phase of breathing, heterogeneous inspiratory excitatory impulses add one to another in a similar way. Consequently it seems that quantity of impulses or more specifically, the summation of depositions of lingering acetylcholine into a smoothed effect becomes the critical factor in reflex excitation just as the amount of extraneous acetylcholine injected is the factor determining the intensity of response. Is it not advisable to look for neuro-architectural machinery in which the pattern of *structure* rather than pattern of *impulses* exercises the dominating influence on motor integration?

The first prerequisite in this concept is the proof that the respiratory center is capable of responding in a rhythmical way to a steady drive. That was provided many years ago by the continuance of rhythmical breathing after dorsal root deafferentation of the respiratory center (Marckwald, 1888) and more recently by the continuance of rhythmical discharges in the phrenic nerve after immobilization by curari (Winterstein, 1911; Bronk and Ferguson, 1935).

The second prerequisite is the proof that the discharge of the respiratory center robbed of its normal periodic afferent drive still has the same general basic pattern of activity of a physiologically intact animal. Here, too, the answer is positive (Gesell, Atkinson and Brown, 1940) for action potential studies have demonstrated that the activity of the respiratory center during curari poisoning is not only rhythmical but is organized in co-ordinated details. It must follow that steady drives which motivate breathing, regardless of their source (chemoceptors, nociceptors, proprioceptors, etc.) are converted by the inherent structure of the respiratory center into geometric patterns of motor activity characteristic of normal breathing. This pertinently conforms with the requirements of neurohumoral integration, for a relatively slow rate of destruction of acetylcholine is adaptable for a fusion of incoming impulses into a common steady drive. The actual "pooling" of acetylcholine, therefore, becomes an integral part of nervous integration.

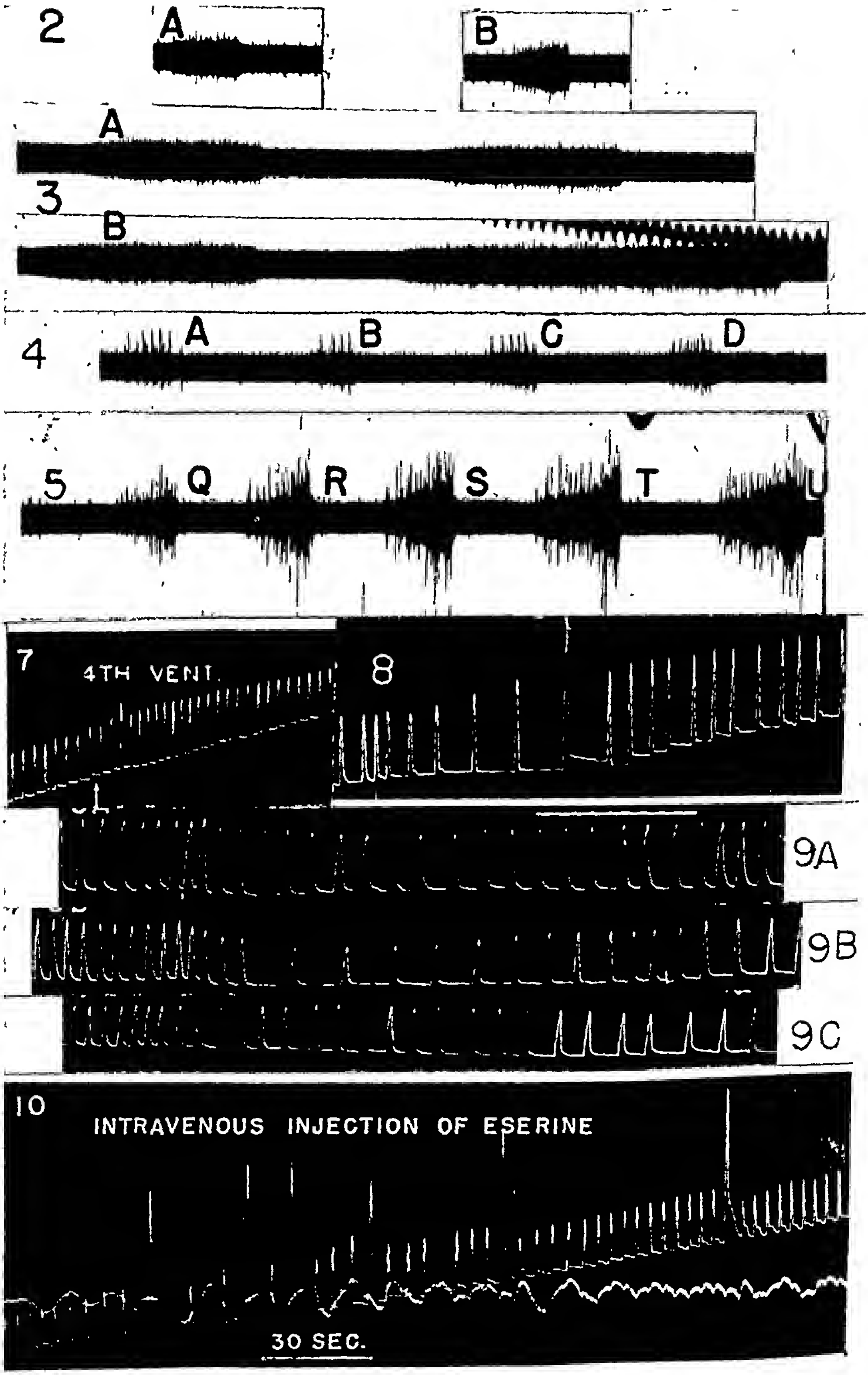
The third step in the concept of structural dominance in motor integration

consists in determining the basic activity pattern of the response of the respiratory center to the shapeless stimulation provided by arterial injection of extrinsic acetylcholine. Specifically—are the geometrical activity patterns of normal breathing retained or are they lost in acetylcholine hyperpnea? The answer to this question is found in part in figures 2, 3, 4, 5 and 6.

5. *Proof of the basic similarity of physiological hyperactivity of the respiratory center and that produced by extrinsic acetylcholine.* The slowly augmenting inspiratory pattern of eupnea is not only preserved but actually intensified by acetylcholine hyperpnea. (Compare fig. 2B with 2A, 3B with 3A and 5Q, R, S, T and U with 4A, B, C and D. The fusillades in figs. 2 and 3 are recorded from the phrenic nerve and those in figs. 4 and 5 from respiratory muscles.) It must be recalled that the characteristic triangular configuration of the normal eupneic inspiratory pattern shows a progressively increasing activity during the course of each individual inspiration and that the increasing amplitude of the electrical record is the combined result of an increasing frequency of activity (either of nerve cells or muscle fibers) and of a progressive recruitment of newly activated units (Gesell, Atkinson and Brown, 1941). Both factors lead to a growing summation of increasingly coinciding potentials which reaches a maximum at the end of the inspiratory act.

The accentuation of the inspiratory activity pattern (fusillade) by arterial injection of acetylcholine is evidence in itself that the standard integration of normal inspiratory activity is employed by the respiratory center in extrinsic humoral hyperpnea. Note the increasing fusillades in figures 5Q, R, S, T and U and compare with the eupneic fusillades in figures 4A, B, C and D. But if the electrodes are fortunately situated in a relatively inactive portion of a muscle, they will register the individual activity of one or more newly recruited units and reveal step by step the manner in which eupnea is intensified and the characteristic activity pattern retained during hyperpnea produced by the central action of acetylcholine (see figs. 6A to M).

When breathing is relatively stable, as it commonly is after vagotomy, the performance of individual muscle units is also relatively dependable, to the extent that an incompletely recruited muscle unit will begin to twitch at an appointed moment and at an appointed increasing twitch frequency schedule, and that it will deliver a relatively uniform number of twitches per inspiration, let us say 7 as in figure 6A. If physiological hyperpnea were now to be produced that particular unit would immediately contribute to the respiratory response in several ways. It would begin to twitch longer and at a higher frequency and it would deliver a greater number of twitches per inspiration. While this unit was progressively reaching its maximum activity other newly recruited units would join the contraction at the very end of inspiration and go through a similar process. The same general scheme of integration holds for hyperpneas produced by the central action of acetylcholine. This is illustrated in figures 6A, B, C and D where 0.12 mgm. of acetylcholine is injected into the carotid artery of a chemoceptively denervated dog. The effects come on in less than two seconds and therefore are unquestionably central. The electrical changes of the internal



Figs. 2, 3, 4, 5, 7, 8, 9, 10

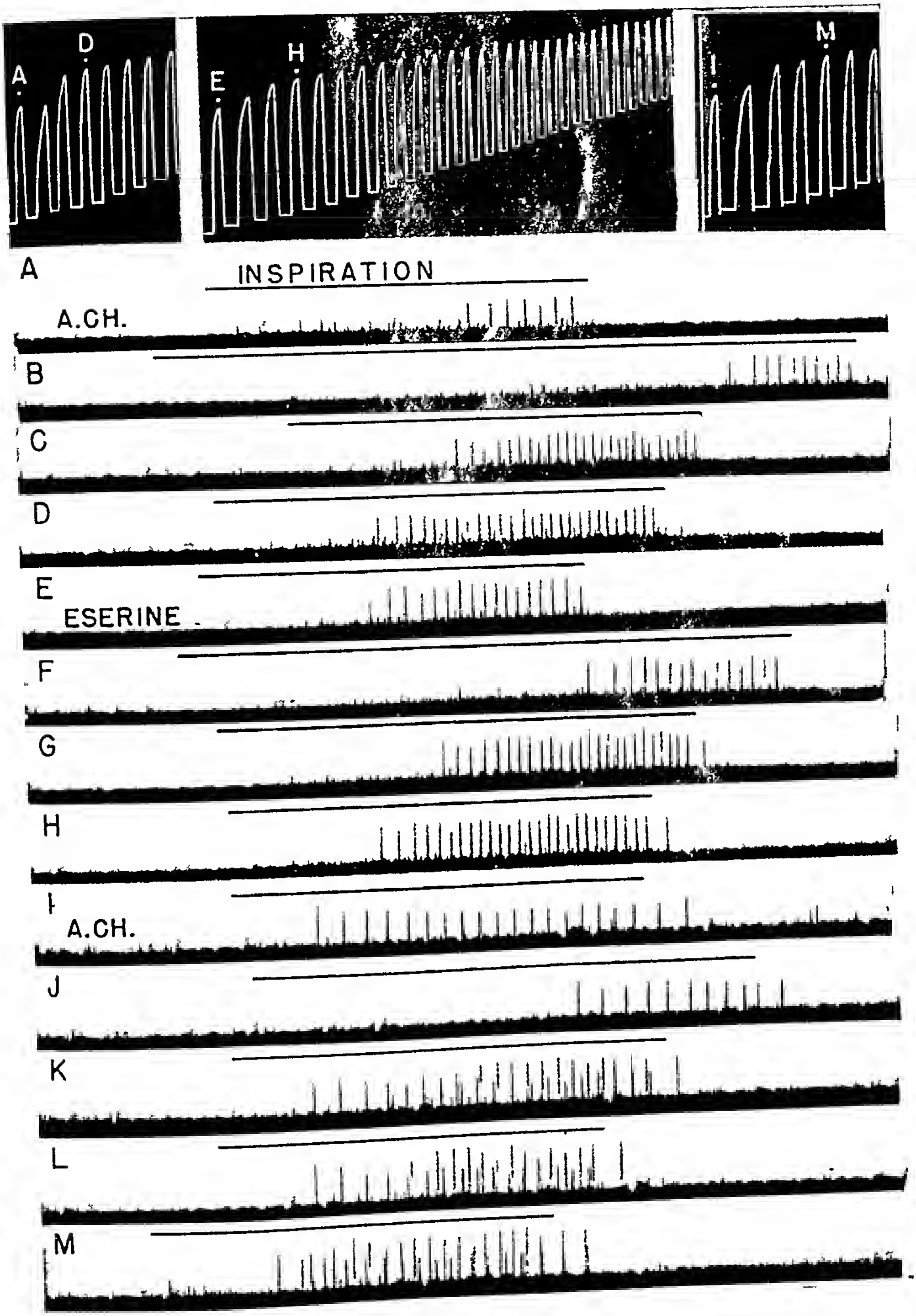


Fig. 6

intercostal muscle were recorded on a rapidly moving paper to give better definition to the potentials. The smoked record which runs much more slowly is, however, suited to show the changes in depth of breathing and is included in the

figures. The discrete potentials superimpose themselves upon a background fusillade of slowly developing intensity (very faintly visible). The appearance of the discrete twitches in the latter third of normal inspiration 6A is evidence that the normal mechanism of recruitment of muscle units is at work to meet the need of increasing mechanical energy of that individual inspiration, and respirations 6B, C and D, appearing immediately after acetylcholine show the extension of the normal mechanism of recruitment into hyperpnea. The incompletely recruited unit still speeds its pace with each inspiration in hyperpnea as it did in eupnea. It twitches for a longer period and at a higher maximum frequency and in respiration 6B it delivers 10 tugs instead of 7. Often the first inspiration of hyperpnea develops very slowly and by its prolongation tends to throw the recruited unit off its regular schedule when judged by the moment of onset of twitching, but that disturbance disappears in the next inspiration, 6C. Here the activity begins much earlier in the inspiratory phase, it lasts twice as long, and delivers 2.6 times as many twitches, which means an appreciable increase of gross frequency of twitch. The maximum frequency of twitch is higher than it is in 6B, and approximately twice that of the control inspiration 6A.

Figures 6E, F, G, and H show that the same mechanism of gradation of hyperpnea is operative when eserine (0.4 mgm.) instead of acetylcholine is injected into the carotid artery. The prolonged and slow development of the first inspiration after eserine (6F) disturbs the schedule as it did above, but in the following inspirations this unit responds in a manner typical for acetylcholine. It twitches over a longer portion of the inspiratory phase, and at a higher maximum twitch frequency and it delivers approximately 27 tugs as compared with the initial 17.

In figures 6I, J, K, L and M there are two units whose activity can be followed. There is a completely recruited unit which during eupnea (fig. 6I) contracts from the very beginning of inspiration and through the entire inspiratory phase. There is also an incompletely recruited unit which becomes active in the second breath after acetylcholine, 6K. This unit can be distinguished from the completely recruited unit by its taller electrical deflection. As is usual for this animal, the activity schedule of the muscle units is disturbed in the first prolonged inspiration after injection. The total number of twitches delivered by the completely recruited unit falls from 20 to 11, but in the next breath which is of the same duration as that of eupnea, twitching is back to schedule (20 per inspiration). That agrees with our concept that a completely recruited unit cannot be pushed by increasing stimulation beyond a set limit, which in turn agrees with the decrease of the number of twitches of this unit as the duration of the succeeding inspiration shortens. The incompletely recruited unit however answers to the increasing respiratory drives in the usual way. In breath 6K it begins to twitch only after its fully recruited mate had delivered its 7th twitch, in breath 6L it becomes active after the 5th twitch, and in breath 6M it has almost reached complete recruitment. It now joins the contraction after the second twitch of its mate. In breath 6K it contributes 14 tugs, in breath 6L which is considerably shorter it nevertheless delivers the same number of twitches which is indicative of increased activity. In breath 6M, which is also short, it delivers a total of 18

twitches. It is apparent that these two muscle units have followed the orderly rules of integration which are expected in physiological hyperpnea.

We have the evidence that expiratory muscular activity is intensified by acetylcholine and eserine in the same manner as is the activity of the inspiratory muscles. The triangularis sterni which contracts in the slowly augmenting way, characteristic of all inspiratory muscles, shows an intensification of activity and of pattern like that illustrated in figures 5Q, R, S, T and U, and 6A to M. It remains only to state that we have also abundant electrical data showing a highly co-ordinated intensified interaction of the half-centers in which the frequency of alternation of activity is greatly augmented in conjunction with augmented intensity of discharge of the individual half-centers. The electrical records are the counterpart of what might be expected in figure 1H where the spirometer tracing clearly indicates greater power and greater frequency of inspiratory and expiratory contractions.

It is, therefore, suggested that the electrical data on the respiratory muscles contain the direct evidence required to establish the duplication of central co-ordinated nervous integration common to physiological hyperpnea by extrinsic acetylcholine.

6. *Some effects of acetylcholine placed on the floor of the fourth ventricle.* Sensitivity of the fourth ventricle to chemicals (Nicholson, 1936; Nicholson and Sobin, 1938) recommends localized application of minute quantities of acetylcholine and eserine as employed by Miller on the cortex. Simple hyperpnea is the common effect (fig. 7). Sometimes breathing is intensified but retarded (fig. 8) as it is by intravenous injection of eserine (fig. 10). Both inspiration and expiration are strengthened, the latter holding inspiration in abeyance and accounting for the prolonged expiratory phases. These results suggest a central potentiation of the dual excitatory vagal proprioceptive reflexes for the vagi are intact. The breathing resembles that resulting from sustained inflation of the lungs which is known to increase the dual excitatory drive of the vagus nerve (Gesell, Brookhart and Steffensen, 1937; Gesell, 1940). These analogous effects indicate that intensification of stretch receptor activity by pulmonary inflation and potentiation of normal unintensified stretch receptor reflexes by extrinsic acetylcholine are equivalent phenomena.

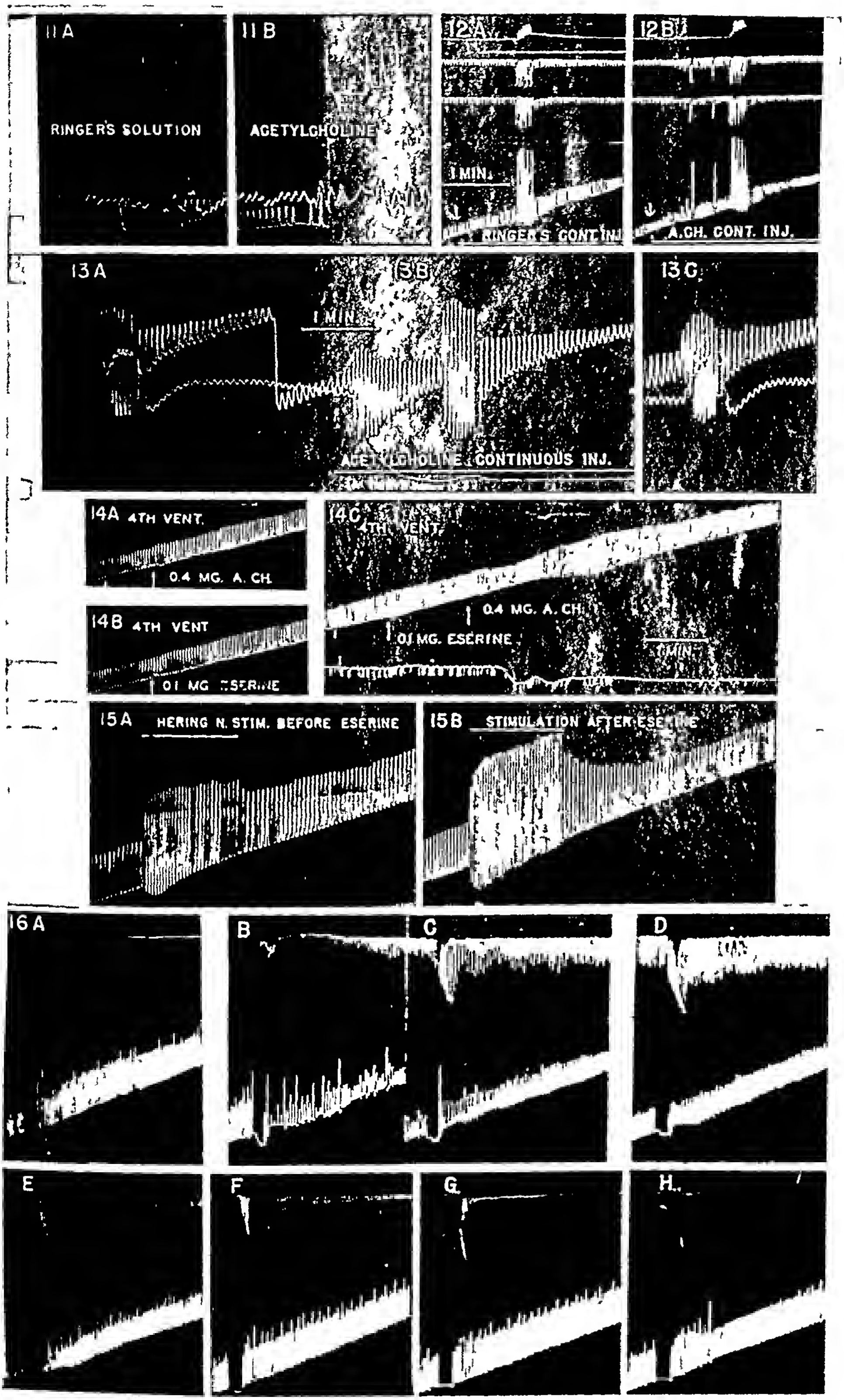
In figure 9A where the vagi are cut, the powerful inspiratory excitatory reflexes of the vagi are missing. The effects are predominantly expiratory.

7. *The similarity of effects of extrinsic and intrinsic acetylcholine.* Sporadic deep breaths and high frequency of breathing (figs. 1A, B, H and I and 12B) are rather characteristic effects of extrinsic acetylcholine in vagally intact animals whereas a slower and more even type of breathing in which inspirations are markedly prolonged is more peculiar to the effects of acetylcholine in vagotomized individuals (see figs. 1D and F). The respiratory response to chemical stimulation of the carotid body or to faradic stimulation of Hering's nerve (both of which in the light of the evidence of this paper releases intrinsic acetylcholine) is modified in the same way by the elimination of the vagal reflexes (Gesell, 1940). Note the prolongation of second inspiration in figure 3B by acetylcholine. This indicates

that the administration of extrinsic acetylcholine is comparable in its effects with those of a continuous release of intrinsic acetylcholine by faradic stimulation of Hering's nerve. We believe that either extrinsic or intrinsic acetylcholine is capable of providing comparable steady states of stimulation and that both potentiate and consequently interact in similar ways with incoming impulses. Thus, if powerfully stimulated inspirations (Hering nerve stimulation or extrinsic acetylcholine) are unsupported by the inspiratory excitatory vagal reflex and its accompanying inspiratory interrupting component (expiratory excitatory drive) inspirations tend to be deep but slow (compare figs. 1D and 1E, 1G and 1I, and 1F and 1H). If on the contrary the inspiratory excitatory reflex and its interrupting component are intact, inspirations tend to be swift and brief and of uneven depth (see figs. 1B, H and I).

8. *The central potentiation of reflexes by acetylcholine.* Dale and his associates have regarded potentiation of indirect stimulation or of extrinsic acetylcholine by eserine or prostigmine as an important step in the proof of humoral intermediation in all outlying cholinergic systems (including the sympathetic and parasympathetic ganglia). Various attempts made from time to time to establish potentiation in central humoral intermediation have met with less convincing results than those on the simpler systems where potentiation is now accepted as fact. The uncertainty rests on the variability of findings. A single reflex is found to be either strengthened or weakened, or one particular reflex is found to be intensified while another is paralyzed. This is hardly surprising or contradictory if it be remembered that the nerve cells of the central nervous system are continuously bombarded by impulses of varying and unknown number, that acetylcholine is capable of producing diametrically opposite effects (stimulation or paralysis depending upon its concentrations), and that inhibition of one reflex may be the expression of excitation of another reflex entirely overlooked.

The observation that acetylcholine produces rapid and forceful breathing when supported by the highly excitatory vagal proprioceptive reflexes (figs. 1H and I) was interpreted above as a possible example of potentiation. More direct evidence for this important phenomenon was sought with artificially induced reflexes. Figures 11A and 11B show the effects of 1.5 mgm. of acetylcholine in Ringer's solution placed on the floor of the fourth ventricle upon the respiratory response to pulmonary inflation. The lungs were inflated by weighting a specially constructed steel spirometer, floating on mercury (Gesell and Moyer, 1935), which allows a continuous recording of breathing. The retardation of breathing during pulmonary inflation attended by marked intensification of the inspiratory act and prolongation of the expiratory phase (11A and 11B) is evidence of an active stimulation of the vagal proprioceptive reflexes. Plain Ringer's solution before inflation of the lungs had no effects upon breathing and, therefore, presumably none during inflation. Acetylcholine in Ringer's solution, however, called forth a preliminary retardation of breathing of its own and a prolonged compression of the lungs (witnessed by the downward stroke of the spirometer tracing) and a sharp increase in the inspiratory excursions. This retardation of breathing before pulmonary inflation is interpreted as a dominant expiratory excitation holding inspiration in abeyance through reciprocal in-


11 A
RINGER'S SOLUTION
11 B
ACETYLCHOLINE
12A
1 MIN.
RINGER'S CONT. INJ.
12B
A.CH. CONT. INJ.
13 A
13 B
1 MIN.
ACETYLCHOLINE CONTINUOUS INJ.
13 C
14A 4TH VENT.
0.4 MG. A. CH.
14B 4TH VENT
0.1 MG ESERINE
14C 4TH VENT.
0.4 MG. A. CH.
0.1 MG. ESERINE
1 MIN.
15A HERING N. STIM. BEFORE ESERINE
15B STIMULATION AFTER ESERINE
16 A
B
C
D
E
F
G
H

Figs. 11–16

hibition. The greater retardation of breathing during increased pulmonary inflation is regarded as a potentiation of the dominating expiratory reflex.

Figures 12A and B show potentiation of hyperpnea produced by faradic stimulation of right Hering's nerve. Chemical stimulation of the carotid bodies was avoided by appropriate crushing of both Hering's nerves. The vagus nerves were left intact. Acetylcholine evoked an increase of hyperpnea of approximately 35 per cent (10 instead of 7 deep breaths). Much greater potentiation is not uncommon. The heightened rhythm of the breathing is interpreted as a potentiation of the vagal proprioceptive reflexes which are normally so active in driving both half centers and whipping up the frequency of breathing.

In figures 13A, B and C both vagus nerves as well as both Hering's nerves were cut. Hering's nerve was then stimulated for periods of 30 seconds before, during, and after injection of acetylcholine into the vertebral artery. In contrast to figure 12 in which stimulation of Hering's nerve had vagal proprioceptive support the effects of potentiation were on the amplitude rather than on the frequency of breathing. This increase in amplitude was primarily inspiratory.

To sum up: Figure 11 illustrates potentiation by extrinsic acetylcholine of an expiratory reflex, figure 13 a disproportionate potentiation of an inspiratory reflex, and figure 12 a relatively even potentiation of inspiratory and expiratory activity because the frequency of breathing is increased.

9. *Central potentiation of the respiratory response to extrinsic acetylcholine by eserine.* Figures 14A, B and C show the potentiating effects which eserine (placed on the floor of the fourth ventricle) had on the respiratory response to similar placement of 0.4 mgm. of acetylcholine. Before eserine (14A) acetylcholine had no apparent effects. After two placements of 0.1 mgm. of eserine (14B and C) which by themselves had no effects, 0.4 mgm. of acetylcholine produced a definite increase of breathing (14C).

Figure 9B shows a potentiation by eserine of a predominantly expiratory effect produced by acetylcholine on the floor of the fourth ventricle after double vagotomy. Compare with the effects of acetylcholine before eserine in figure 9A.

10. *Central potentiation of artificial reflexogenic stimulation by eserine.* Normally faradic stimulation of the chemoceptor afferents is expected to increase the depth of both inspiration and expiration illustrated in figure 15A (Gesell, 1940). This probably depends upon the simultaneous impingement of chemoceptor impulses at both the inspiratory and expiratory half-centers. Deeper inspirations and deeper expirations produced by stimulation of Hering's nerve after eserinization (fig. 15B) point to a potentiation of each of the dual excitatory effects of the chemoceptor impulses, i.e., to a greater accumulation of intrinsic acetylcholine at the individual half-centers.

Figure 16A to H shows the effects of stimulation of the superior laryngeal nerve before and after injecting 1 mgm. of eserine (stimulation at intervals of about 1, 3, 8, 18, 23, 42, and 58 min. after injection). The usual expiratory compression of the lungs (see spirometer tracing) agrees with the predominantly expiratory excitatory effect of this nerve. This compression was increased in succeeding stimulations B, C, D and E, for a period of 23 minutes following eserinization. Following record F potentiation of this reflex disappears.

The response of the facial muscles to stimulation of the superior laryngeal nerve shows even more striking potentiation (downstroke inspiration). During eupnea (fig. 16A) the response is weak and subsides quickly. One minute after eserine (fig. 16B) the response is slightly greater, and in the next ten stimulations there is a rapid, powerful, rhythmical response followed by prolonged after-potentiation. These effects decrease slowly and vanish within one hour after the injection of eserine. The prolonged "after-hyperpnea" in the facial muscles following stimulation is interpreted as an "after-discharge" caused by excessive pooling of highly protected acetylcholine.

The appearance of accessory respiratory contractions of the facial muscles in record B is most probably an expression of a slowly developing potentiation of the physiological reflexogenic drives of these muscles resulting from the prolonged effects of eserine.

Discussion. The reproduction of physiological activity in the respiratory center through the intermediation of extrinsic acetylcholine is significant in several ways. First of all it differs from the reproduction of activity in the outlying cholinergic systems (nerve muscle units, e.g.) in its composite nature, i.e., in the interaction of interrelating parts (true nervous integration). Contrast the peripheral with the central. A motor nerve impulse releases acetylcholine at the motor end plate and produces a single twitch in the corresponding muscle unit. Such twitches are reproducible by close and rapid arterial injections of a minute quantity of acetylcholine. Tetanic contractions can also be reproduced either by a massive or a prolonged administration of acetylcholine. So long as threshold concentrations of acetylcholine exist, the muscle will respond repeatedly to this steady stimulus. Such reproductions of activity are but examples of physiological stimulation in its simplest forms and are scarcely to be regarded as demonstrations of nervous integrations.

On the other hand when extrinsic acetylcholine acts upon the respiratory center a composite of highly integrated changes in nervous activities develops. 1. An increased frequency of alternation of inspiration with expiration. 2. An increased depth of inspiration. 3. An increased depth of expiration. 4. Earlier interruption of inspiration. 5. Earlier interruption of expiration. 6. A more rapid acceleration of inspiratory muscle twitching during the course of inspiration. 7. A more rapid and greater recruitment of active inspiratory units. 8. A more rapid acceleration of expiratory muscle twitching during expiration. 9. A more rapid and greater recruitment of active expiratory units during expiration. 10. A most probable increased intensity of reciprocal inhibition between half-centers for the increased intensity of the forces of stimulation at both half-centers must be held in reciprocal check.

All of these changes are the result of an exceedingly crude stimulation which has but two attributes—quantity and duration. Thus neuro-architectural structure was concluded to be of paramount importance in breathing. The question of the relative importance of architectural and sensory pattern is frequently raised and notably by Weiss (1941), who has shown that transplanted sections of the spinal cord exhibit rhythmical activity which is augmented by increased activity of the host, suggesting a similarity to experimental hyperpnea produced by extrinsic acetylcholine.

While the volume and the receptor source of afferent impulses are of undoubted value in motor integration it is nevertheless pertinent to realize that the inherent architectural machinery of the respiratory center and its inherent activity can and does actually determine the pattern of inflowing signals (Gesell, 1940). For example, the progressively augmenting activity of the inspiratory center during each inspiration stretches the lungs, increases the discharges of the stretch receptors, and augments the inspiratory excitatory component of the stretch reflex thereby intensifying the inherent inspiratory activity which is in progress. Inherent central activity and centrally motivated proprioceptive reflexes are consequently of the same progressively augmenting pattern and therefore build smoothly one into the other. Thus vicious cycles must develop which require co-ordinated machinery to interrupt the pyramiding activity. Logically one looks to simultaneous pyramiding stimulation at the opposing half-center to accomplish this end.

The factor of mass stimulation is in favor of the predominating rôle of neuro-architecture for in the greater response of the respiratory center to greater quantities of acetylcholine the typical pattern of nervous activity is retained or even intensified.

All findings seem to fit the view that acetylcholine potentiates the effects of acetylcholine. This potentiation may be merely the result of a simple addition of acetylcholine at the site of stimulation. In order that this phenomenon of summation (accumulation) may play a rôle in nervous integration it is essential that a fraction of the acetylcholine released by a nerve impulse should outlive the interval between impinging signals. The greater the sum total of impinging impulses the greater should be the accumulation of acetylcholine. Consequently the myriads of impulses composing the inspiratory excitatory reflex should increase in power by virtue of the pyramiding effects of increasingly large residual fractions of acetylcholine accumulating at increasing frequencies as the rate of discharge of the stretch receptors mounts. Thus greater power is very simply provided as it is needed.

Pooling of acetylcholine at the cardinal points in any nervous circuit becomes the paramount tool of nervous activity. Control of pooling becomes the means of gradation, lack of control the source of inco-ordination. Robbed of its normal supply of impulses a nervous circuit must labor under disadvantage.

Granted that humoral intermediation of nervous integration has been demonstrated in the respiratory act it seems fair to assume that a similar mechanism functions in *all* nervous integrations. On that point we extend the logic of Forbes (1939) who stated "if it is finally established that intercellular transmission is chemically mediated in such widely different systems as neuro-muscular junctions of smooth and striated muscle and the synapses of ganglia, the operation of a wholly different mechanism in the histologically similar synapses of the central grey matter would be a most surprising anomaly".

SUMMARY AND CONCLUSIONS

Central neuro-humoral nerve cell activation was studied on the respiratory act of the dog.

It was found that acetylcholine produced hyperactivity of the respiratory

center whether injected intra-arterially or applied to the floor of the fourth ventricle.

This effect was in relation to the concentration of acetylcholine at the center as revealed by the graded response to graded injection and by the greater effect of intravertebral than intracarotid injections after denervation of the carotid and aortic chemoceptors.

The activity produced was essentially a normal hyperpnea showing the characteristic series of changing and co-ordinated events (about ten in all) during a respiratory cycle.

The response to acetylcholine was modified by the concurrent balance of incoming impulses as shown by the difference with intact and blocked vagi.

Acetylcholine injections (extrinsic acetylcholine) had an additive effect to that liberated by incoming impulses (intrinsic acetylcholine) as shown by combining either Hering nerve stimulation or the lung inflation reflex with acetylcholine injections.

Eserine potentiated the action of acetylcholine (extrinsic and intrinsic) as shown by the increased stimulation of acetylcholine deposited on the floor of the fourth ventricle and the greater and more prolonged reflexogenic response to faradic stimulation of the superior laryngeal and Hering's nerve. Potentiation of activity was demonstrated in both half-centers.

Because the crude and shapeless chemical stimulation produced by central injection of acetylcholine evoked a highly co-ordinated activity it was concluded that neuro-architectural patterns rather than sensory patterns of impinging impulses exercise the dominant rôle in nervous integration.

It was proposed that the basic conclusions reached in these studies on the respiratory act are applicable to the central nervous system in general.

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THE EFFECT OF PANCREATECTOMY ON FAT ABSORPTION FROM THE INTESTINES[1]

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The recent development by Whipple and his associates (1) and by Brunschwig (2) of methods for the radical removal of carcinoma of the ampulla of Vater and of the pancreas has emphasized anew some old problems concerning the relation of this organ to the digestion and absorption of fat in the intestines. It is now well established that the exclusion of pancreatic juice from the intestines by pancreatectomy or by successful occlusion of the pancreatic ducts produces a marked impairment in the absorption of fat and proteins. The early observations of Pratt and his associates (3) have been confirmed by many workers in this field. Coffey, Mann and Bollman (4) studied the fecal excretion of fat in normal, pancreatectomized dogs, and animals in which the pancreatic juice had been excluded from the intestines by ligation of the pancreatic ducts or by external pancreatic fistula. In the normal animal only 2 to 4 per cent of the ingested fat was recovered in the feces, whereas in the other preparations from 54 to 76 per cent of the fat fed was not absorbed. Confirming the observations made by many others these workers also reported that from one-third to one-half of the unabsorbed fat appeared in the feces in the form of free fatty acids. According to Nothmann and Wendt (5), however, this splitting of the fat occurs chiefly in the large intestine presumably through bacterial action and the fat in the upper small intestine is largely unhydrolyzed. When a fatty acid such as oleic acid was fed to depancreatized dogs, normal absorpton took place, i.e., only 13 to 34 per cent appeared in the feces. If confirmed, these observations would account for the defect in fat absorption after pancreatectomy exclusively on the basis of failure of digestion of the fat of the food by the pancreatic lipase in the duodenun and jejunum.

In the present investigation, a study was made of the absorption of a neutral fat (olive oil) and its corresponding fatty acid (oleic acid) in pancreatectomized dogs. Normal dogs were used as controls and only such pancreatectomized animals as had completely recovered from the operation and were in otherwise good health were used in the experiment. Two methods were used to study absorption. In the first method, analysis of the blood for total lipids was done at intervals after the administration of the test fat. In the normal animal a characteristic lipemic curve is known to occur following fat feeding. We, therefore, investigated the effect of administration of both olive oil and oleic

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acid on the blood lipids in normal control and in pancreatectomized dogs. In a second experiment, the amount of fat absorbed was determined by a study of the fat in the feces after the feeding of a measured amount of olive oil or oleic acid.

Absence of alimentary hyperlipemia in pancreatectomized dogs. Healthy dogs weighing from 7 to 12 kgm. were operated upon and the entire pancreas removed. Postoperatively insulin was given to all dogs twice daily. The insulin dosages were adjusted to limit glucose excretion to 4 to 6 grams in 24 hours. Fat absorption tests were not begun until the dogs had recovered from the operation and were eating well. Food was then withheld for a period of 18 to 48 hours during which time no insulin was given. As a control for subsequent studies the blood lipids for the 12 hours following the fasting period were determined at 3 hour intervals on four dogs which were given no fat. The blood lipids in these dogs showed no striking change throughout the twelve hour period and satisfied us that if no fat is given the blood lipids remain relatively constant in pancreatectomized animals in the fasting state. The effect of administration of fat upon the blood lipids was then studied. In the first experiments 50 cc. of olive oil were given by stomach tube, but the administration of oleic acid by stomach tube caused most of the dogs to vomit within a short time. Therefore, other means of introducing the fat were sought. Under anesthesia (ether or nembutal), with the abdomen open, the fat was injected directly into the duodenum in repeated small quantities with a needle and syringe. But even in normal animals lipemia did not occur. We believed that this was an effect of the anesthesia. Accordingly a method was devised to enable direct injection of the fat into the duodenum in the absence of anesthesia. This was done by inserting a de Pessar catheter into the duodenum, bringing the end of the catheter out through the abdominal wall. After complete recovery from the operation the dogs were then fasted. On the morning of the test oleic acid was injected through the catheter in small quantities until 50 cc. had been given over a period of an hour.

Blood was drawn from the femoral vein at the time of fat administration and at frequent intervals thereafter for eight to twelve hours. Serum lipids were determined by a modification of Bloor's method, previously described from this laboratory (6). The results obtained are summarized in figure 1. The initial or control serum lipid is plotted at the zerobase line and subsequent determinations appear as deviations above or below this level. The solid line curve for normal dogs represents the average obtained in two animals and shows that the blood lipid rises to a maximum of 330 mgm. per cent three hours after the oral administration of 50 cc. of olive oil and then gradually declines. The solid line curve for pancreatectomized dogs represents the average of at least one experiment on each of eighteen animals. In no instance did hyperlipemia occur. The dotted line curve summarizes experiments on four depancreatized dogs and shows that the administration of pancreatic juice does not restore the normal hyperlipemia due to olive oil feeding. These animals were given 200 cc. of fresh active pancreatic juice by stomach tube twice daily for seven days and

then on the morning of the absorption test 200 cc. of juice was given along with the olive oil. The broken line curve represents the average of one experiment on each of six depancreatized dogs and shows that raw pancreas is equally without effect in restoring the normal hyperlipemia in response to olive oil feeding. One hundred grams of fresh raw pancreas was fed daily to each of these animals for a week before the absorption test was made.

The data summarized in figure 2 are somewhat surprising in that they show that the absorption of fatty acids is no better than for neutral fat after removal of the pancreas. The solid line curve indicates that in three normal dogs a definite hyperlipemia within three hours was produced by the instillation of 50 cc. of oleic acid into the duodenum. No such hyperlipemia was secured in the depancreatized animals even with the feeding of raw pancreas.

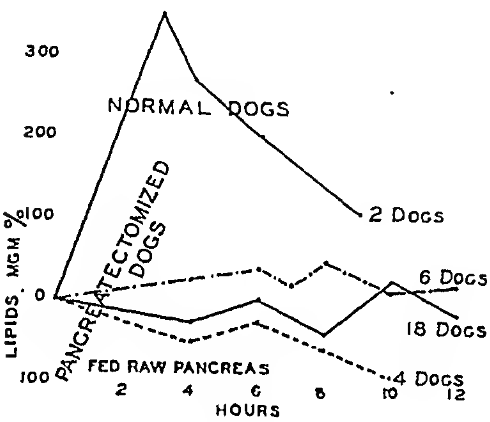

Fig. 1

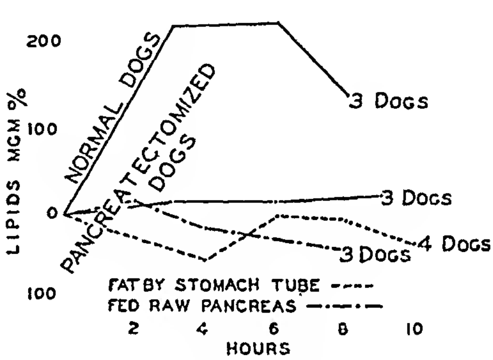

Fig. 2

Fig. 1. Average deviation of blood lipids from 0 hour level after administration of 50 cc. olive oil by stomach tube to normal and pancreatectomized dogs.

Fig. 2. Average deviation of blood lipids in dogs from 0 hour level after administration of 50 cc. oleic acid to unanesthetized normal and pancreatectomized dogs. Oleic acid instilled into duodenum per catheter except in one series of 4 pancreatectomized animals in which it was given by stomach tube.

Absorption of neutral fat and fatty acid in depancreatized dogs as determined by analyses of the feces. In this experiment pancreatectomized dogs which had recovered from operation were placed on a "fat-free" diet containing potatoes, carrots, casein, sucrose, skimmed milk, brewer's yeast and defatted bone meal. On this diet each animal obtained only 8 grams of fat per day. After two to three days on this "fat-free" diet the animals were given olive oil well mixed with the basic meal. After a period of two to four days the oil addition was discontinued and only the basic diet given. Then again for a period of two to four days oleic acid was added to the diet. In some animals the procedure was reversed, the oleic acid being given first and olive oil in the second period. The addition of carmine to the food at the beginning of each change of feeding made it possible to collect the feces corresponding to the periods of olive oil or oleic acid feeding with reasonable accuracy. The defatted bone meal was added to the food to produce stools sufficiently formed for easy collection. Any food left uneaten was weighed and subtracted from the total weight given and cor-

rection made for the amount of oil actually eaten. In some instances this calculated uneaten fat was checked by actual extraction of fat in the Soxhlet apparatus. The stools of each dog for an entire period were combined, a fine suspension in alcohol was prepared in a rotary mixer, and an aliquot of the suspension was dried. The fat was extracted with alcohol and alcohol-ether in a Soxhlet apparatus. The fat was re-extracted with petroleum ether and weighed after drying. Checks on the fat content of the food were similarly made.

TABLE 1

DOG	TIME AFTER OPERATION	FEEDING PERIOD	TOTAL FOOD FAT	OLIVE OIL TAKEN	OLEIC ACID TAKEN	STOOL FAT	PER CENT ABSORPTION OILVE OIL	PER CENT ABSORPTION OLEIC ACID
Comparative absorption olive oil and oleic acid in pancreatectomized dogs								
		days	*grams*	*grams*	*grams*	*grams*	*per cent*	*per cent*
1	1 yr.	4	29.3	342		143.3	61.5	
		4	27.8		241	110.5		58.9
2	2 mo.	4	28.6	305.2		80	76.0	
		4	26.6		182	53.5		74.4
3	1 yr.	4	29.3	171		133.8	33.0	
		4	29		151	77.5		57.0
4	3 mo.	3	17	110.6		70.8	46.0	
		3	14		91	16.9		83.6
5	4 mo.	3	20	128.6		127.2	14.2	
		3	20		126	95.8		35.4
6	1 mo.	3	15	101.3		71.7	38.3	
		3	20		126	43.9		70.0
7	1 mo.	3	15	98.0		44.0	61.1	
		3	20		126	47.2		67.8
						Average	47.2	63.7
Comparative absorption olive oil and oleic acid in normal control dogs								
8		4	29.3	171		1.9	99.05	
		4	29.3		151	11.0		93.9

The data are presented in table 1. The normal animal absorbed 99 per cent of the olive oil and 94 per cent of the oleic acid fed. This agrees very well with the results of other investigators. In the pancreatectomized dog, however, considerable variation was found. One animal absorbed 76 per cent of the olive oil, another only 14.2 per cent, and the remainder amounts intermediate between these extremes. Similar variations occurred in the absorption of oleic acid, i.e., from 83.6 to 34.4 per cent of the amount fed. In three of the animals there was no appreciable difference between the absorption of olive oil and oleic acid. In the remaining four, however, significantly better absorption of oleic

acid took place, i.e., 33 versus 57 per cent, 46 versus 83 per cent, 14 versus 34 per cent, and 38 versus 70 per cent.

Discussion. According to one theory of fat absorption widely held at the present time (7) glycerides in the food are emulsified by the bile and split into fatty acids and glycerol by lipolytic enzymes derived chiefly from the pancreatic juice. The fatty acids then form a water soluble, freely diffusible, combination with bile salts and enter the intestinal mucosa in this form. The compound with bile salts is then broken up and the fatty acid is set free. Glycerol passes independently into the intestinal mucosa, recombines with the fatty acids, and the neutral fat thus formed passes into the lymph. The impairment in fat absorption following pancreatectomy and the fact that fatty acid is on the whole somewhat better absorbed than neutral fat, are in harmony with this view. Nevertheless, the remarkable absorption of neutral fat in some depancreatized dogs which may amount to from 61 to 76 per cent of the fat in the diet, indicates either that this hydrolysis is not essential or else that sufficient lipase is furnished by the gastric and intestinal juices. Improvement in fat absorption with longer survival after operation is not apparent in our data. The decided impairment in the absorption of fatty acid after pancreatectomy is somewhat surprising and may indicate that the pancreas plays some rôle in the absorption of fat other than through the digestive action of pancreatic lipase.

The disappearance of the normal hyperlipemia in response to the oral administration of neutral fat or fatty acid in depancreatized dogs is doubtless due to the impaired and perhaps delayed absorption indicated by the studies on the fat in the feces. This finding may possibly have some significance in the diagnosis of pancreatic disease.

CONCLUSIONS

1. Temporary hyperlipemia, which may be produced in normal dogs by the oral administration of neutral fat or fatty acid, is abolished by removal of the pancreas and is not restored by the administration of active pancreatic juice or raw pancreas.
2. Pancreatectomy produces a varying degree of impairment in the absorption of neutral fat, but some animals may still absorb 75 per cent or more of the fat in the diet.
3. Pancreatectomy produces a definite impairment in the absorption of fatty acid, though not so great as in the case of neutral fat.

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